REMARKS

I.   The Subject Matter of the Claims

The subject matter of the claims relates, in general, to methods of modulating the transport of leptin across the blood-brain barrier (BBB).

II.   Claim Objections.

The Examiner objects to claims 3-5 as assertedly being of improper dependent form for failing to further limit the subject matter of a previous claim. The Examiner asserts that recitation of leptin variants, analogs, fusion proteins, etc. in claims 3-5 is broader in scope than the term "leptin" used in claims 1 and 2. Applicant respectfully disagrees.

The doctrine of claim differentiation necessitates that when two claims in the same patent have an apparently similar or identical meaning, an effort should be made to adopt an interpretation that will give them a different (as distinguished from identical) meaning. Under the doctrine of claim differentiation, each claim in a patent is presumptively different in scope. RF Delaware Inc. v. Pacific Keystone Technologies Inc., Fed. Cir., 2003. The difference in meaning and scope between claims is presumed to be significant "[t]o the extent that the absence of such difference in meaning and scope would make a claim superfluous." Tandon Corp. v. United States Int'l Trade Comm'n, 831 F.2d 1017 (Fed. Cir. 1987).

From the order of the claims and in keeping with the doctrine of claim differentiation, it is clear that Applicant means "leptin" in claim 1 to be inclusive of all derivatives of leptin set out in dependent claims 3-5. In addition, page 4, lines 15-18, of the specification describes that "leptin" includes analogs, fragments, consensus leptin, chemical derivatives or fusion proteins. In order to expedite prosecution of the application, claims 3 and 4 have been amended to clarify the leptins contemplated for use in the methods of the claims. The Applicant have also revised

11

claims 3-4 to be in proper Markush format. As such, the Examiner's objection is moot and

Applicant requests that the objection be withdrawn.

III.   Patentability Argument

A.   The Rejection of Claims 1-5 Under 35 U.S.C. §112, First Paragraph, for Lack of Enablement Should Properly Be Withdrawn

The Examiner maintains the rejections of claims 1-5 as assertedly not being enabled

because, according to the Examiner, Applicant has not enabled transport of leptin across the

blood-brain barrier (BBB) using all the compounds recited in the claims, by every route of

administration in the claims, and using all said compounds co-administered with all leptins,

leptin derivatives and fragments thereof. The Examiner asserts that there are "so many non-

working embodiments disclosed in the specification" that the claims are not enabled over the

claimed scope. The Examiner further asserts that because an exemplified embodiment may fall

into more than one class of compounds in the claims, the claims are equivalent to a single means

claim, which may be rejected for having undue breadth since the structure or functional class of

the claimed genus has assertedly not been enabled. Applicant respectfully disagrees.

"The test [for undue experimentation] is not merely quantitative, since a considerable

amount of experimentation is permissible, if it is merely routine, or if the specification in

question provides a reasonable amount of guidance with respect to the direction in which the

experimentation should proceed to enable the determination of how to practice a desired

embodiment of the claimed invention." Johns Hopkins Univ. v. Cellpro, Inc., 152 F.3d 1342, 47

U.S.P.Q.2D 1705 (Fed. Cir. 1998). "The enablement requirement is met if the description

enables <u>any</u> mode of making and using the invention." <u>Johns Hopkins Univ.</u>, *supra*. (emphasis added).

The requirement for enablement is whether or not a person of ordinary skill could make and use the invention and not whether each compound is effective in the method of the invention. As stated in Applicant's previous response (dated June 30, 2005), Applicant has enabled use of <u>at least one</u> member of each class of compounds recited in the claims by teaching how to measure leptin transport across the BBB in the presence and absence of compounds of each class recited in the claims (see, for example, Example 1, beginning on page 17, and Example 4, beginning on page 22). Because Applicant has taught compounds useful in the invention and methods for using these compounds to achieve the goal of the method of the invention, Applicant provides a reasonable amount of guidance with respect to how the skilled person should proceed to practice the invention; to wit: all that the skilled worker needs to do is repeat the detailed methods performed with the exemplified compounds using another compound from the same class. This does not amount to undue experimentation. Indeed, it would be a matter of routine and logic to try the next compound in the genus to clarify whether it has the desired effect. In this manner, a worker of ordinary skill can readily ascertain which disclosed compounds are effective in the method based on the description in the disclosure, and as such, one of ordinary skill is not forced to experiment unduly to arrive at the subject matter of the invention. This is nothing more that routine screening following the directions given in the specification.

The Examiner specifically points out that certain members of each class of compounds do enhance leptin transport, while some members of the class do not enhance leptin transport. Applicant reiterates that, as admitted by the Examiner, the specification enables at

13

least one member of each class of compounds recited in the claims that is effective in modulating

transport of leptin across the BBB. The presence of inoperative embodiments within the scope

of a claim does not necessarily render a claim nonenabled. Applicant is not required to

demonstrate efficacy of every member of a certain class of compounds, but enable the class such

that one of ordinary skill in the art could make and use the invention. Atlas Powder Co. v EI du

Pont de Nemours & Co., 750F2d 1569, 224 USPQ 409. Experimentation, even if extensive, is

not necessarily undue if it is routine in the art. In re Wands, 858 F.2d 731 (Fed. Cir. 1988).

In re Wands involved screening of large numbers of hybridomas to identify specific

hybridomas that fell within the claim limitations. The court in Wands indicated that because

Wands provided sufficient guidance to make and screen the hybridomas and presented working

examples, the enablement requirement was fulfilled. In re Wands, 858 F.2d 731, 740 (Fed. Cir.

1988). In re Wands does not hold that a specific number of working examples is required. In

reaching its decision, the court in Wands considered that the inventor's disclosure provides

considerable direction and guidance on how to practice the invention and presents working

examples. Id at 740. When such provided guidance is coupled with high level of skill in the art,

the invention is enabled. Id. This is exactly the case in the present application.

Applicant provides guidance in the specification as to what compounds are members

of each class of agents and also teaches methods for determining if a compound is a modulator of

leptin transport. A worker of ordinary skill could take any compound, whether it is disclosed in

the application or not, and test for its ability to act as a leptin transport modulator. For example,

it would be routine for a person of ordinary skill to take an adrenergic agonist, neurotransmitter

or cytokine not named in the specification but well-known and readily available in the art (see

Section III.B below) and assess its function in the methods described in Example 1. It requires

no more than routine screening of compounds in a standard experimental mouse model disclosed in the specification, similar to the routine screening in the Wands case, to arrive at the present invention.

Furthermore, it is understood in the art that while species within the class of, for example, neurotransmitters or adrenergic antagonists, may not have a strong structural relationship, these species clearly have a functional relationship as evidenced by the understanding of these terms in the art (See Principles of Internal Medicine, Isselbacher et al., Eds. 13th edition, 1994, Ch. 364, "Impact of neurobiology and molecular genetics on neurology," page 2210 and Table 68.1) (included herewith in Exhibit A). One of ordinary skill can readily find reference to, description of, and members of the genus of compounds (e.g., adrenergic agonists, adrenergic antagonists, neurotransmitters, cytokines, amino acids, opiate peptides, purinergic agonists, glutaminergic agonists) in the art (see Borges et al., *supra,* which refers to the genus of adrenergic drugs, including agonists and antagonists, see abstract). Thus, the specification has enabled the genus of compounds as they are commonly identified by the class of compound they represent, and provides sufficient guidance for one of ordinary skill to understand what is taught by the classification of the compounds.

With respect to the Examiner's objection to the enablement of leptin analogs, variants, derivatives and fragments thereof, Applicant respectfully disagrees, but nonetheless has amended the claims in order to expedite prosecution, and the claims as amended are directed to leptin, consensus leptins, leptin fusion proteins, chemically modified derivatives of leptin, and fragments of leptin. As stated previously, all of these leptins are enabled in the specification and in the art. For example, U.S. Patents 6,734,160 and 6,471,956 (submitted with the response of June 30, 2005 as Exhibit A) describe and claim leptin fragments and analogs. These fragments

15

are described in terms of where the leptin protein may be truncated or substituted (see US Patent

6,471,956, col. 18 to col. 20). The art also describes methods to determine if a leptin sequence

has biological activity, by measuring leptin binding to leptin-specific antibodies, leptin

competition assays, and leptin receptor binding assays, all routine experiments regularly

performed by one of ordinary skill.

Moreover, the specification, at page 11, lines 1-27, describes consensus leptins and

leptin fragments useful in the methods of the invention, and also teaches, at page 12, line 15 to

page 16, line 5, leptin fusion proteins and leptin derivatives having chemical moieties (e.g.,

PEG). The specification teaches one of ordinary skill in the art how to make a leptin fragment or

consensus leptin, teaches that leptins, including fragments and consensus leptins, as

contemplated by the invention retain the biological activity of modulating weight or altering

metabolism in a host mammal (page 9, lines 11-13), and also teaches methods for measuring

transport across the blood-brain barrier (see Example 1). Further, mouse models for measuring

transport across the BBB, such as the one used in the Examples in the specification and in

Borges et al, are well-known and readily available to one of ordinary skill. As such, a worker of

ordinary skill in the art could make and use a leptin contemplated for use in the invention

without undue burden using the guidance taught by Applicant.

Peelman (*J Biol Chem* 279:41038-46, 2004) is cited as an example of unpredictability

in the art, assertedly demonstrating that a single amino acid change may alter the activity of a

leptin analog or fragment. The claims in the present application are not directed to specific

leptin compositions but to methods of making a more effective use of leptins. Leptins are

known. The problem being solved in the present invention is increasing the uptake of such

leptins across the BBB. Whether or not a new, as yet unknown, leptin analog is predictable or

not is not relevant to the question. The more appropriate question is would there be

unpredictability in conducting the *methods* of *this* invention. Applicants posit that the answer to

that question is no. Rather than demonstrating unpredictability, Peelman shows that the skilled

person may successfully make numerous leptin variants and successfully test all such variants for

leptin function without undue burden (page 41041-2). This demonstrates that one of ordinary

skill in the art can readily make leptin variants and screen them for the desired biological

activity, such as binding to the leptin receptor and modulating of metabolism. Thus, the leptins

may be produced and screened without undue experimentation, as evidenced by Peelman.

Therefore, the field of leptin chemistry is not unpredictable, and the specification, in connection

with the knowledge in the art, provides guidance to make and use the leptin contemplated for use

in the methods of the invention. Applicant reiterates that the subject matter removed from the

claims is enabled by the specification, and has been removed solely to expedite prosecution.

Applicant reserves the right to pursue this subject matter in a continuing application.

The Examiner asserts that Applicant's interpretation of In re Fischer is incorrect.

Applicant submits that the interpretation is not Applicant's alone, but was recited at MPEP

2164.01(b), "as long as the specification discloses at least one method for making and using the

claimed invention that bears a reasonable correlation to the entire scope of the claim, then the

enablement requirement is satisfied. In re Fischer, 427 F2d 833, 166 USPQ 18 (CCPA 1970)."

The compounds contemplated in the claims bear a strong correlation to the compounds

exemplified in the specification for use in the method of the invention. The leptin fragments or

derivatives contemplated by the invention retain the same activity as the leptin exemplified in the

specification, while the compounds contemplated to modulate transport across the BBB are

additional species of the compounds exemplified in the specification.

17

With respect to the routes of administration of the compounds, Applicant has amended claims 3 and 4 to remove reference to intracerebrovascular and intracisternal administration to expedite prosecution.

With respect to the other routes of administration allegedly not enabled by the specification, Applicant submits that these methods of administration are routine in the art and known to allow entry of administered molecules (including leptin) into the systemic circulation. For example, Ciulla et al (*Retina* 25:619-24, 2005) describe that the compound linezolid administered orally was detectable in the serum of patients; Huang et al (Adv Drug Deliv Rev 29:147-55, 1998) describe that nasal administration of compounds is an attractive method for mediating systemic administration of large molecules; Becit et al (*Eur J Vasc Endovasc Surg* 22:310-6, 2001) describe that VEGF given intramuscularly or intraarterially improves angiogenesis in subjects. (All abstracts included herewith in Exhibit B) Other routes of administration, including subcutaneous, intradermal, and topical administration, are identified in art-recognized pharmaceutical treatise as standard routes of drug administration that are effective at mediating systemic effect of the agent being administered (see Remington: the Science and Practice of Pharmacy, 19th Edition, Mack Publishing Company, Easton, Pa. (1995), Ch. 41, pp. 710-12, Exhibit B). In addition, MPEP 2164.01(c) states that "if a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 USC §112 is satisfied." As such, the routes of administration taught in the specification, which are well-known and routine in the art, are fully enabled by the specification.

Applicant cited In re Borkowski [422 F2d 904, 164 USPQ 642 (CCPA 1970)] to address the Examiner's objection to the term "modulating", and not to address undue

experimentation in general as suggested by the Examiner. As such, the Examiner's note that In re Borkowski is not germane does not apply in the context used by the Examiner.

The Examiner states at page 4 of the Action that claim 1 is equivalent to a single means claim and as such should be rejected under 35 USC 112, but offers no explanation of how claim 1 is equivalent to a single means claim and why, even if there is some comparison to a single means claim, why the claim necessarily must be rejected. Examiner cites MPEP 2164.08(a) as support. MPEP 2164.08(a) refers to In re Hyatt (708 F.2d 712, 218 U.S.P.Q. 195 Fed. Cir. 1983), which defined a single means claim as a claim which recites merely one means plus a statement of function and nothing else (Id. at 713). The claim at issue in Hyatt was directed to a "processor comprising incremental *means for* incrementally generating... incremental output signals..." (Id. at 712). Applicant submits that the pending claims do not fall into a single means category similar to In re Hyatt as the present claims recite more than one element needed for the method and more than one "means" by which to modulate leptin transport. Applicant is not claiming "every conceivable means for achieving the stated result" (Id. at 714), but is actually claiming a method of using a genus of compounds which is enabled in the specification, and the genus of compounds is one which is well known to those of skill in the art. Applicant is claiming a method comprising more than one element, that uses one or more of a group of compounds that are defined (and known in the art) by their function(s), not a method that cites any means of inducing transport of leptin across the BBB. Applicant submits that Examiner's comparison of the present claims to a single means claim is misplaced.

Because the specification teaches multiple species within a class of compounds contemplated for use in the methods of the invention, discloses methods for determining if a compound modulates leptin transport across the BBB, describes methods to make leptin or a

19

leptin fragment, consensus sequence or derivative, and teaches routes by which the compounds

can be administered, Applicant has taught a worker of ordinary skill in the art to make and use

the methods of the invention. The worker of ordinary skill in the art would only have to use

routine experimentation to repeat these methods. Therefore, the rejection under 35 U.S.C. § 112,

first paragraph, enablement, should properly be withdrawn.

**B.   The Rejection of Claims 1-5 Under 35 U.S.C. §112, First Paragraph, Written
         Description Should Properly Be Withdrawn**

The Examiner maintains the rejection to claims 1-5 as lacking written description

asserting that Applicant has not described all the agents recited in the claims or all leptin variants

and fragments encompassed by the claims. The Examiner asserts that there is insufficient

structure/function correlation described with respect to both the genus of compounds used in the

methods as well as the leptin fragments and derivatives to indicate to a worker of ordinary skill

that Applicant was in possession of the invention. Applicant respectfully disagrees.

With respect to the agents recited in the claim 1, the specification sets out a

representative list of each class of compounds recited in the claim (see page 5, line 3 to page 6,

line 9) and not an exhaustive list of those classes of compounds because they are readily

available in the art. While the species within each genus or class may not share structural

characteristics, they are necessarily defined by their functional characteristics otherwise they

would not be members of the particular genus described. See e.g., Principles of Internal

Medicine, *supra*, which identifies drug types based on agonistic or antagonistic functions (Table

68-1) (Exhibit A).

20

The Examiner points out that structure/function relationship is only one of many

factors to consider, along with functional characteristics, to provide evidence of possession of a

genus (see the Action, page 8). A worker of ordinary skill would understand what is meant by an

adrenergic agonist or antagonist, or cytokine or neurotransmitter when these terms are generally

used in the art and are based on their functional activity (see e.g., Borges et al., *supra*, page 244,

col. 1, which refers to adrenergic agonist or antagonist; and, Houseknecht et al., *J Anim. Sci.*

76:1405-20, 1998, page 1406, col. 1 and abstract, which refers to cytokine and neurotransmitter

respectively) (Exhibit C).

What is conventional or well known to one of ordinary skill in the art need not be

disclosed in detail. MPEP 2163 citing <u>Hybridtech v Monoclonal Antibodies, Inc.</u>, 802 F2d 1367,

231 USPQ 81 (Fed Cir. 1986). A worker of ordinary skill can readily access the art to easily find

a list of compounds falling within the well-known class of compounds recited in the claims. See

<u>Principles of Internal Medicine</u>, *supra*. Applicant's disclosure of representative members of each

genus of compounds, coupled with the general knowledge in the art, demonstrates that a person

of ordinary skill would readily understand that Applicant was in possession of the claimed genus

of compounds.

With respect to the assertion that leptin fragments and derivatives are not adequately

described, Applicant stated previously that biologically active leptin fragments, variants,

consensus sequences and the like are well know in the art (See U.S. Patents 6,350,730,

6,309,853, 6,734,160, 6,429,290 and 6,471,956). Moreover, the specification describes specific

leptin fragments, variants, consensus sequences, fusion proteins and derivatives contemplated for

use in the method of the invention (page 10, line 1, to page 12, line 8). The specification recites

which sequences are optimal in a leptin fragment polypeptide, which amino acids can be

21

changed to arrive at a consensus sequence and to which amino acids they can be changed. The

specification also describes that the leptin may have 83% or more identity to the leptin sequences

recited in the claims (page 12, lines 1-2).

The claims also recite that the leptin is biologically active, which is taught in the

specification as the ability to modulate weight or modulate metabolism (page 9, lines 11-13). A

worker of ordinary skill understands that the biological function of leptin is mediated through the

leptin receptor (Karonen et al., *Eur J. Nucl Med* 25:607-12, 1998, abstract submitted herewith in

Exhibit C). Binding to the receptor is hypothesized by those of ordinary skill to mediate

transport across the BBB (Meister B., *Vitam Horm* 59:265-304, 2000, abstract submitted

herewith in Exhibit C). As such, the structure/function correlation of a leptin derivative useful in

the method of the invention is that the leptin retains biological function, which necessitates that

any leptin derivative retain binding to its receptor, and is thereby able to be transported across

the BBB. Only routine screening is required to determine if a leptin analog, fragment or variant

binds to the leptin receptor (see U.S. patent 6,471,956 and Peelman, *supra*).

Given the description in the specification and the general skill and knowledge in the

art, one of ordinary skill in the art would recognize that Applicant was in possession of the

invention at the time of filing, given the extensive description of specific leptin fragments,

consensus sequences, or derivatives, and the routine methods of screening binding of leptin to

the leptin receptor.

As such, the rejection of claims 1-5 under 35 USC §112, first paragraph, written

description, should be withdrawn.

**C.   The Rejection of Claims 1-5 Under 35 U.S.C. §112, Second Paragraph,
Should Properly Be Withdrawn**

The Examiner objects to recitation of the term "effective amount of exogenous leptin"

as assertedly being indefinite. Applicants initially introduced (i) in the claim so that there would

be appropriate antecedent basis for the term "wherein said administering of step (ii) is effective

to modulate the transport of leptin across the blood brain barrier." Applicants have amended (i)

to recite "administering to the mammal a composition that comprises exogenous leptin" and

believe that this amendment still provides an antecedent basis for (ii) but also obviates the

grounds for the current rejection. Should the Examiner wish to discuss alternative language to

address this rejection, the Applicants respectfully request that the Examiner contact the

undersigned.

**D.   The Rejection of Claims 1-5 Under 35 U.S.C. §103(a),
Should Properly Be Withdrawn**

The Examiner rejected claims 1-5, as amended, under 35 U.S.C. §103(a) as assertedly

obvious in view of the disclosure of Banks, further in view of Borges, further in view of Caro.

The Examiner asserts that because Banks and Caro assertedly teach that leptin requires transport

across the BBB, and Borges assertedly teaches that epinephrine increases permeability of

molecules across microvascular cells in vitro, a worker of ordinary skill in the art would be

motivated to combine the teachings of Banks, Caro and Borges to arrive at the present invention.

Applicant respectfully disagrees.

Banks teaches administration of leptin to a mammal to suppress food intake. Banks

neither discloses nor suggests administration of epinephrine to increase leptin transport. Caro

assertedly discloses that leptin levels are correlated with body mass index and obesity, and

23

indicates that leptin likely crosses the BBB using a saturable transport mechanism. Caro

suggests that administration of exogenous leptin would be ineffective because leptin must cross

the BBB via a transport mechanism. Caro neither discloses or suggests the administration of

epinephrine in conjunction with leptin.

Borges allegedly teaches that administration of epinephrine can increase the

permeability of microvascular endothelial cells to impermeable solutes, exemplified by sodium

fluorescein bound to albumin. Borges neither discloses nor suggests administration of leptin in

conjunction with epinephrine to increase leptin transport. Moreover, Borges utilizes a molecule

that exhibits **natural** diffusion across the BBB through a mechanism other than transport

mechanisms. Figure 1 of Borges (page 245, col. 1) shows that the agent of interest naturally

diffuses across the cell membrane, and the rate of diffusion increases over time.

Common uses of epinephrine relate to promotion of peripheral vascular resistance in

cardiac arrest and also as a bronchodilator in asthma, due to its characteristic non-specific action

on adrenergic receptors (See Principles of Internal Medicine, Isselbacher et al., Eds. 13th edition,

1994, page 420 and Table 68.1) (included herewith in Exhibit D). However, epinephrine is also

a non-specific disruptor of the BBB allowing diffusion or leakage of molecules across the

membranes (Sokrab et al., *Acta Neurol Scand.*, 77:387-96, 1988, abstract submitted herewith in

Exhibit D). Administration of epinephrine can lead to permanent neuronal damage and even

death in subjects receiving the agent. (Sokrab et al., *supra*). Epinephrine typically acts to

increase blood pressure in a subject and is used as a model for hypertension (Sokrab et al.,

supra).

Applicants submit that the Examiner is in error in combining the teachings of Banks

and Caro with the teachings of Borges as the two papers are discussing entirely different

24

mechanisms of transport. Banks and Caro teach that leptin requires a *specific* transport

mechanism to transport exogenous leptin across the BBB. In contrast, Borges teaches that

epinephrine *non-specifically* increases the permeability of molecules across an artificial BBB

model, as well as affects many other pathways in a subject. A person of ordinary skill reading

Borges would not reasonably expect that an agent that non-specifically increases BBB

permeability would cause a specific transport leptin across the BBB via the specific transport

system discussed in Banks and Caro. Therefore, disclosure of a specific transport system for

leptin in Banks and Caro would not motivate a worker of ordinary skill to look to the teaching of

Borges, which teaches a non-specific transporter such as epinephrine, to arrive at the methods of

the present invention.

Indeed, Applicants believe that the cited art suggests a teaching away from the

methods claimed in the present invention. Borges teaches that epinephrine has many non-

specific effects in the body, including disrupting the BBB, and can allow any molecule to diffuse

across the BBB in an uncontrolled manner. As such, one of ordinary skill would have no

motivation to administer epinephrine in conjunction with any molecule that requires specific

uptake across the BBB, because epinephrine would cause a random non-specific diffusion of all

molecules. There is certainly no indication in Borges, or Banks and Caro, that epinephrine may

also cause a specific uptake of any molecule. Given this lack of teaching in the art, there would

be no reasonable expectation of success at obtaining the specific transport of leptin as being

claimed in the present invention. One of ordinary skill would reasonably expect that

administration of epinephrine would disrupt the BBB to such an extent that any molecule would

diffuse across the membrane and therefore leptin would be just one of many molecules crossing

25

the BBB, thereby negating the specific effect desire by the administration of leptin disclosed herein.

In view of the art teaching away from use of epinephrine to specifically induce transport across the BBB, Banks and Caro do not provide motivation to look to Borges, and vice versa, to arrive at the present invention. One of ordinary skill would have no motivation to arrive at the present invention based on the disclosures of Banks, Borges and Caro. Therefore, the rejection of claims 1-5 under 35 USC §103 should properly be withdrawn.
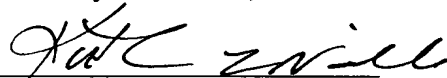
IV.   Conclusion

No fees are believed due in connection with the filing of this response, however, should any fees be deemed necessary, the Commissioner is hereby authorized to deduct any such fees from Marshall, Gerstein and Borun LLP account number 13-2855.

Applicants submit that the application is now in condition for allowance and respectfully request notice of the same.

Dated: October 18, 2005

Respectfully submitted,

By_____
Katherine L. Neville, Ph.D.
Registration No.: 53,379
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Driver, Suite 6300
Sears Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Agent for Applicant

26

THIRTEENTH EDITION

HARRISON'S
PRINCIPLES
OF
INTERNAL
MEDICINE

Editors

KURT J. ISSELBACHER, A.B.

Mallinckrodt Professor of Medicine, Harvard Medical
School; Physician ard Director, Cancer Center,
Massachusetts General Hospital, Boston

EUGENE BRAUNWALD, A.B.

Hersey Professor of the Theory and Practice of Medicine,
Harvard Medical School; Chairman, Department of
Medicine, Brigham and Women's Hospital, Boston

JEAN D. WILSON

Charles Cameron Sprague Distinguished Chair and
Professor of Internal Medicine; Chief, Division of
Endocrinology and Metabolism, The University of Texas
Southwestern Medical Center, Dallas

JOSEPH B. MARTIN, M.D., Ph.D.,
F.R.C.P. (C), M.A. (Hon.)

Professor of Neurology and Chancellor, University of
California, San Francisco

ANTHONY S. FAUCI, M.D.

Director, National Institute of Allergy and Infectious
Diseases; Chief, Laboratory of Immunoregulation; Director,
Office of AIDS Research, National Institutes of Health,
Bethesda

DENNIS L. KASPER, M.D.

William Ellery Channing Professor of Medicine, Harvard
Medical School; Chief, Division of Infectious Diseases,
Beth Israel Hospital; Co-Director, Channing Laboratory,
Brigham and Women's Hospital, Boston

Note: Dr. Fauci's work as editor and author was performed outside the scope of his employment by the U.S. government. This work represents his personal and professional views and not necessarily those of the U.S. government.

Thirteenth Edition

34567890   DOW DOW   98765

TABLE 364-1 Molecular genetics of neurologic disorders *(continued)*

Chromosome location	Disorder	Principal clinical findings/Phenotype	Mode of inheritance	Genotype/gene product	Genetic testing?	Ref. (or Chap.)
Xq27.3	Fragile-X syndrome	Decreased head size, prominent forehead, large ears, macro-orchidism, mental retardation.	X-LR	Gene characterized by trinucleotide 5' repeats of CGG. Gene identified as *FMR-1*.	Yes.	62 and 378
Xq28	Adrenoleukodystrophy	Mild neuropathy, spastic paraparesis, baldness, hypogonadism, hypoadrenalism.	X-LR	Gene characterized.	Possible.	377
Xq28	Emery-Dreifuss muscular dystrophy	Onset before age 10, benign course characterized by slowly progressive proximal weakness, cardiac arrhythmias may occur.	X-LR	Unknown.	Not available.	385

Abbreviations: AD. autosomal dominant; AR. autosomal recessive; X-LR. X-linked recessive; HPRT. hypoxanthine-guanine phosphoribosyltransferase.
SOURCE: Martin JB. Ann Neurol. vol. 34. 1993.

tion that dopamine cells are affected in Parkinson's disease led to therapy with levodopa. In Huntington's disease, it has been possible to define the subsets of neurons affected by the degenerative process in the striatum and to show selective sparing of other cell types (see Chap. 370). Although this has not led yet to specific theories about the mechanisms of cell death, it has resulted in speculations about potentially beneficial therapeutic strategies.

Many of these newly discovered neuropeptides appear to share nerve terminals and often even secretory vesicles with conventional neurotransmitters. It appears likely that neuropeptides exert profound modulatory effects on the actions of the primary neurotransmitter. In other cases the peptide may influence neuronal plasticity, growth, or differentiation. A most surprising discovery was the recognition that nitric oxide (NO), synthesized in neurons by the enzyme nitric oxide synthase, acts after cellular release to exert a variety of effects on neural tissues. First recognized as an endothelial cell–derived muscle cell relaxant (nitrates dilate blood vessels, as in the treatment for angina), NO can be both beneficial and toxic to brain cells. Subclasses of neurons contain NO synthase, as for example do NADPH diaphorase-positive neurons of the striatum, and NO release in normal quantities can activate other neurons. Excessive release can prove toxic. Other gases, such as carbon monoxide, may also serve modulatory roles in nervous tissue.

BIOCHEMICAL CLASSIFICATION OF RECEPTOR SUBTYPES Some of the most important insights from neurobiology have resulted from the cloning of the genes of several neurotransmitter and hormone receptors that are critical to brain function. Based on pharmacologic analysis alone it was difficult to account for the diverse effects mediated by molecules of low molecular weight. For example, pharmacologic analysis of acetylcholine disclosed muscarinic and nicotinic effects. With the biochemical elucidation of acetylcholine receptor subtypes and by means of molecular cloning techniques and molecular probes, it is now established that multiple forms of muscarinic (M1, M2, M3, M4, and M5) and nicotinic (N1 and N2) receptors exist in the brain and that their distribution varies from region to region. Thus, differences in receptor subtypes expressed in different regions of the brain can account for the complex multiple effects of the medications acting on cholinergic receptors. The molecular specificities of the various receptor subtypes provide sensitive systems with which to search for selective agonists and antagonists. For example, M1 agonists may facilitate memory. Regional differences in the distribution of the subtypes of the nicotinic cholinergic receptor in the central nervous system make it possible to explore the neurologic basis of nicotine addiction.

Identification of the subclasses of the alpha- and beta-adrenergic, serotoninergic, gamma-aminobutyric acid (GABA), and glutamatergic receptors makes it possible to correlate structure and function and

provide a rational basis for molecular classification of drug actions. In the case of the GABA receptor, structural features that account for the interaction of barbiturates and benzodiazepines can now be recognized.

Subclasses of glutamate receptors mediate excitatory amino acid neurotransmitter effects. Glutamate, one of the most abundant of all neurotransmitters in the brain, functions to promote rapid neurotransmitter depolarization by opening membrane channels that permit diffusion of sodium and potassium ions. These rapid effects are mediated by two receptor subtypes, identified by ligand binding with kainate and AMPA. The identification of an additional subtype of glutamate receptor which binds *N*-methyl-D-aspartate (the *NMDA receptor*) made possible identification of additional glutamate functions. The NMDA receptor appears to mediate other functions that heretofore were classed in the category of plasticity, a process considered important, for example, in memory and learning. The NMDA subtype of glutamate receptor is linked to a voltage-sensitive channel that responds to repetitive activation by the opening of an ion channel. The actions of calcium permit transduction of electrical events into molecular changes that can alter neuronal function permanently, i.e., change cellular function to subserve a memory or learning response.

Based on definitive experimental results, it is now clear that activation of the NMDA receptor can also have deleterious effects on the cell, whereby calcium entry induces *neurotoxicity* that, if sufficiently severe, can lead to neuronal cell death. This mechanism may explain some of the extensive neuronal cell damage that occurs in ischemia, hypoxia, epilepsy, and, perhaps, neurodegenerative diseases (see Choi).

These findings have resulted in great interest in the development of new drugs that might selectively block the NMDA receptor, thereby minimizing the effects of ischemia or hypoxia that occur in stroke or after cardiorespiratory arrest. The profound potential of this work is illustrated by the demonstration that NMDA receptor blockade induced even several hours after the neuronal insult may be "neuroprotective."

Cloning of cellular membrane channels (sodium, potassium, calcium) also has had a profound effect on defining mechanisms of neuronal excitability. It is anticipated that these findings will lead to the discovery of more effective drugs for epilepsy, neuroprotection, and migraine.

BRAIN IMAGING The development of computed tomography and proton imaging by magnetic resonance has revolutionized our ability to define lesions in the brain and spinal cord (see Chap. 365). Other techniques have been developed to make it possible to study brain function as well as structure by the application of positron emission tomography (PET), single photon emission computed tomog-

TABLE 68-1 Some commonly used autonomic drugs[a,b,c]

Agent	Indication	Dose and Route	Comment
ADRENERGIC AGONISTS[d]			
Epinephrine	Anaphylaxis	100–500 µg SC or IM (0.1–0.5 mL of 1/1000 solution of hydrochloride salt); 25–50 µg IV (slowly) every 5–15 min; titrate as needed	Nonselective alpha and beta agonist; increases BP, heart rate Bronchodilation
Norepinephrine	Shock Hypotension	2–4 µg of NE base/min IV; titrate as needed	Alpha and beta$_1$ agonist Vasoconstriction predominates Extravasation causes tissue necrosis; infuse through IV cannula
Isoproterenol	Cardiogenic shock Bradyarrhythmias AV block Asthma	0.5–5.0 µg/min IV; titrate as needed Inhalation	Nonselective beta agonist Increases cardiac rate and contractility (beta$_1$) Tachycardia limits usefulness Dilates bronchi (beta$_2$); cardiac stimulation also occurs
Dobutamine	Refractory CHF Cardiogenic shock	2.5–25 (µg/kg)/min IV	Selective beta$_1$ agonist with greater effect on contractility than heart rate; a congener of dopamine but not a dopaminergic agonist
Phenylephrine	Hypotension	40–180 µg/min IV	Selective alpha$_1$ agonist; useful in antagonizing hypotension of spinal anesthesia
	Supraventricular tachycardia	150–800 µg slow IV push	Pressor effect induces vagotonic response; do not exceed 160 mmH systolic BP
Terbutaline	Asthma	2.5–5.0 mg PO tid; 0.25–0.5 mg SC; inhalation every 4–5 h	Selective beta$_2$ agonist; beta$_1$ (cardiac) effects at higher doses
Albuterol	Asthma	2.0–4.0 mg PO tid or qid; inhalation every 4–6 h	Selective beta$_2$ agonist; beta$_1$ effects (cardiac) at higher doses
Isoetharine	Asthma	Inhalation every 2–4 h	Selective beta$_2$ agonist; some beta$_1$ effects
Metaproterenol	Asthma	10–20 mg PO tid or qid; inhalation every 3–4 h	Selective beta$_2$ agonist; some beta$_1$ effects
Pirbuteral	Asthma	Inhalation every 4–6 h	Selective beta$_2$ agonist; some beta$_1$ effects
Ritodrine	Premature labor	100–350 µg/min IV; 10–20 mg every 4–6 h PO	Selective beta$_2$ agonist; hypokalemia, hyperglycemia, hypotension, cardiac stimulation may occur Neonatal hypoglycemia, hypocalcemia reported
DOPAMINERGIC AGONISTS			
Dopamine	Shock	2–5 (µg/kg)/min IV (dopaminergic range) 5–10 (µg/kg)/min IV (dopaminergic and beta range) 10–20 (µg/kg)min IV (beta range) 20–50 (µg/kg)/min IV (alpha range)	Pharmacologic effects are dose dependent: renal and mesenteric vasodilation predominate at lower doses; cardiac stimulation and vasoconstriction develop as the dose is increased
Bromocriptine	Amenorrhea-galactorrhea	2.5 mg PO bid or tid	Selective agonist of dopamine-2 receptor; inhibits prolactin secretion
	Acromegaly	5–15 mg PO tid or qid	Lowers growth hormone in a minority of patients with acromegaly
INHIBITORS OF CENTRAL SYMPATHETIC OUTFLOW			
Clonidine	Hypertension	0.1–0.6 mg PO bid	Selective alpha$_2$ agonist; potentiates central baroreceptor depressor reflex Abrupt discontinuation may result in withdrawal syndrome with rebound hypertension
Methyldopa	Hypertension	250–500 mg PO every 6–8 h	Metabolized by decarboxylation and beta hydroxylation to α-methyl-norepinephrine, a centrally active selective alpha$_2$ agonist
ADRENERGIC NEURON BLOCKING AGENTS			
Guanethidine	Hypertension	10–100 mg PO qd	Concentrated in sympathetic nerve endings; blocks release of NE in response to nerve impulses and depletes NE stores; prominent orthostatic hypotension
Bretylium	Ventricular fibrillation and tachycardia	5 mg/kg IV	In addition to blocking NE release, has direct effect on electrical properties of cardiac muscle
BETA BLOCKING AGENTS[e]			
Propranolol	Hypertension	40–160 mg PO bid (or higher)	Lipophilic, nonselective Dosage highly variable
	Angina Myocardial infarction Arrhythmias	10–40 mg PO tid or qid 60–80 mg PO tid 10–30 mg PO tid or qid; 1–3 mg IV	Prolongs survival post MI
	Hypertrophic cardiomyopathy Pheochromocytoma	20–40 mg PO tid or qid 10–20 mg PO tid or qid; 0.5–2.0 mg IV	After alpha blockade initiated
	Essential tremor Migraine Hyperthyroidism	20–80 mg PO tid 20–80 mg PO bid or tid 10–60 mg PO tid or qid	
Metoprolol	Hypertension	50–200 mg PO bid 100 mg PO bid	Selective beta$_1$ (cardiac), lipophilic Prolongs survival post MI

	Indication	Dose and Route	Comment
β BLOCKING AGENTS (continued)			
	Hypertension	80–320 mg PO qd	Hydrophilic, nonselective; lengthen dosage inter with renal failure
	Angina	80–240 mg PO qd	
	Hypertension	10–30 mg PO bid	Lipophilic, nonselective
	Myocardial infarction	10 mg PO bid	Prolongs survival post MI
	Hypertension	50–100 mg PO qd	Selective beta$_1$, hydrophilic; lengthen dosage in with renal failure
	Hypertension	5–30 mg PO bid	Nonselective, lipophilic with partial agonist acti
	Angina	10 mg PO qid	
	Hypertension	200–800 mg qid	Selective beta$_1$, hydrophilic, partial agonist acti
	Arrhythmias	200–600 mg bid	
	Hypertension	20–40 mg PO qd	Nonselective
	Hypertension	15–20 mg PO qd	Selective β_1, hydrophilic
	Hypertension	2.5–10 mg PO qd	Nonselective, partial agonist activity, hydrophil lengthen dosage interval with renal failure
	Supraventricular tachycardia	50–200 (μg/kg)/min IV after loading dose of 500 μg/kg/min for 1 min	Selective beta$_1$, very short duration of action
ALPHA BLOCKING AGENTS			
Phenoxybenzamine	Pheochromocytoma	10–60 mg PO bid; titrate as needed	Noncompetitive, nonselective alpha blockade
Phentolamine	Pheochromocytoma	5 mg IV (after test dose of 0.5 mg)	Competitive, nonselective alpha blockade
Prazosin	Hypertension	1–5 mg PO bid or tid	Competitive, selective alpha$_1$ blockade
	CHF	2–7 mg PO qid	
Doxazosin	Hypertension	1–16 mg PO qd	Competitive selective alpha$_1$ blockade, long dur of action
Terazosin	Hypertension	1–5 mg PO qd	Competitive, selective alpha$_1$ blockade, long du of action
COMBINED ALPHA-BETA BLOCKING AGENT			
Labetalol	Hypertension	100–1200 mg PO bid; titrate slowly as needed; 20–80 mg IV (by increments up to 300 mg); 2 mg/min by IV infusion	Competitive alpha and beta antagonist with rela more activity against beta receptors
DOPAMINERGIC ANTAGONIST			
Metoclopramide	Diabetic gastroparesis	10 mg PO qid	Competitive dopaminergic antagonist with pron cholinergic agonist activity
	Gastroesophageal reflux	10–15 mg PO qid	
	Antiemetic (cancer chemotherapy)	10 mg IV	
GANGLIONIC BLOCKING AGENT			
Trimethaphan	Hypertensive crisis (aortic dissection)	1–3 mg/min IV	Competitive ganglionic blocker; some direct vasodilating effects; inhibits parasympathetic as sympathetic nervous system
CHOLINERGIC AGENT			
Bethanechol	Urinary retention (nonobstructive)	10–100 mg PO tid or qid; 5 mg SC	M-2 receptor agonist
ANTICHOLINESTERASE AGENTS			
Physostigmine	Central cholinergic blockade	1–2 mg IV (slow)	Tertiary amine; penetrates CNS well; may caus seizures; used to reverse central anticholinerg effects produced by overdose of atropine or t antidepressants
Edrophonium	Paroxysmal supraventricular tachycardia	5 mg IV (after 1.0-mg test dose)	Induces vagotonic response; rapid onset, short duration of action; effects reversed by atropir
CHOLINERGIC BLOCKING AGENTS			
Atropine	Bradycardia and hypotension	0.4–1.0 mg IV every 1–2 h	Competitive inhibition of M-1 and M-2 recepto blocks hemodynamic changes associated with increased vagal tone

[a] Consult complete prescribing information. [b] Doses for children are not given. [c] Only the more common indications and routes of administration are listed.
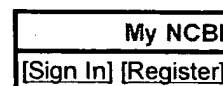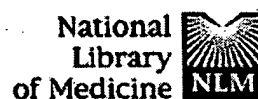
Dopaminergic agonists are listed separately although dopamine, at high doses, is an adrenergic agonist as well.

Clinical efficacy of most beta blockers appears similar for major indications. Not all beta blockers are FDA approved for all indications listed in the table. When beta agents are discontinued, gradual dosage reduction is recommended. Both beta$_1$ selective and nonselective agents have cardioprotective effects after myocardial infarctic

Neuroleptic and antipsychotic agents are also dopaminergic antagonists; these are not included in the table.

A major use of cholinesterase inhibitors is in myasthenia gravis (Chap 386). These agents, quaternary amines that do not penetrate the CNS, are not included here.

A wide variety of synthetic atropine derivatives are available for the purpose of (1) diminishing GI tract motility and secretion and (2) increasing urinary bladder capac usefulness is limited by anticholinergic side effects. Some may be useful as adjuncts in the treatment of peptic ulcer disease.

[Left margin fragments:]
; increases BP.

infuse through

y (beta$_1$)

lation also occurs ffect on ner of dopamine

agonizing

onse; do not

l effects at

cardiac) at

ects
ects

ects hyperglycemia, occur reported

ent: renal and t lower doses: on develop as

r; inhibits

of patients with

ral

hdrawal

ne, a

s; blocks lses and

irect effect

NCBI **Pub Med** National Library of Medicine **NLM** My NCBI [Sign In] [Register]

All Databases   PubMed   Nucleotide   Protein   Genome   Structure   OMIM   PMC   Journals   Books

Search PubMed ▾ for [   ] Go Clear

Limits   Preview/Index   History   Clipboard   Details

Display Abstract ▾ Show 20 ▾ Sort by ▾ Send to ▾

About Entrez

Text Version

All: 1   Review: 0 ✖

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

☐ **1:** Retina. 2005 Jul-Aug;25(5):619-24.   Related Articles, Links

LWWonline
LIPPINCOTT WILLIAMS & WILKINS

Human vitreous distribution of linezolid after a single oral dose.

Ciulla TA, **Comer GM**, **Peloquin C**, **Wheeler J**.

Vitreoretinal Service, Midwest Eye Institute, Indianapolis, Indiana 46280, USA.
thomasciulla@yahoo.com

PURPOSE: To evaluate the relationship between vitreous linezolid concentrations versus both time and serum concentrations after a single 600 mg oral dose. METHODS: Two groups of six subjects undergoing a pars plana vitrectomy indicated by macular pucker or full thickness macular hole were given a single tablet of linezolid before surgery. The early group underwent vitrectomy at random times before the time of maximum serum linezolid concentration (i.e., 77 minutes) and the late group underwent vitrectomy at random times afterward. Each patient had a serum sample drawn shortly before and after vitrectomy and the vitreous specimen was sampled at the initiation of surgery. RESULTS: The early group and late group had mean vitreous linezolid concentrations of 0.06 mcg/mL and 1.25 mcg/mL, respectively. The vitreous linezolid concentration showed a strong correlation to the interpolated serum concentration ($R2 = 0.74$, $P < 0.01$) at the time of vitrectomy. CONCLUSION: The study demonstrates that linezolid penetrates the blood-retina barrier in noninflamed eyes. Because the vitreous concentrations appeared to exponentially trend upward with time and 33% of the late group achieved sufficient MIC90 levels for the common pathogens found in postoperative endophthalmitis, adequate concentrations might be achieved with an altered dosing regimen to achieve higher serum steady state levels. Further study is warranted.

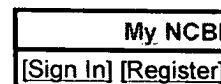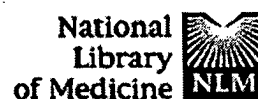PMID: 16077360 [PubMed - indexed for MEDLINE]

Display Abstract ▾ Show 20 ▾ Sort by ▾ Send to ▾

Sep 14 2005 04:34:46

Pub Med

My NCBI
[Sign In] [Register]

All Databases   PubMed   Nucleotide   Protein   Genome   Structure   OMIM   PMC   Journals   Books

Search PubMed for _____ Go Clear

Limits   Preview/Index   History   Clipboard   Details

Display Abstract   Show 20   Sort by   Send to

About Entrez

Text Version

All: 1   Review: 0

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Adv Drug Deliv Rev. 1998 Jan 5;29(1-2):147-155.   Related Articles, Links

ELSEVIER
FULL-TEXT ARTICLE

Large molecule and particulate uptake in the nasal cavity: the effect of size on nasal absorption.

Huang Y, Donovan MD.

The University of Iowa, College of Pharmacy, Iowa City, IA 52242, USA

One of the characteristics influencing the increased interest in the nasal cavity as a site for systemic drug delivery is the ability of large molecules to permeate through the nasal mucosa into the systemic circulation. Compilations of data regarding the absorption of large therapeutic agents, peptides and proteins in particular, along with more systematic studies using polymeric compounds have shown that for compounds larger than 1000 Da, bioavailability can be directly predicted from a knowledge of molecular weight. In general, the bioavailability of these large molecules ranges from 0.5 to 5%. Particulate uptake also occurs in the nasal mucosa, and particles up to approximately 1 microm have been shown to rapidly enter the bloodstream following intranasal administration. The unique barrier properties of this mucosal delivery site give it great promise as a route for the systemic administration of large molecules.

PMID: 10837585 [PubMed - as supplied by publisher]
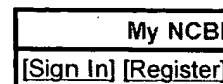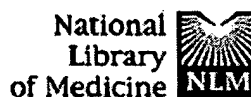
Display Abstract   Show 20   Sort by   Send to

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Privacy Statement | Freedom of Information Act | Disclaimer

Sep 14 2005 04:34:46

NCBI

Pub Med

National Library of Medicine NLM

All Databases   PubMed   Nucleotide   Protein   Genome   Structure   OMIM   PMC   Journals   Books

Search PubMed ▼ for [   ] Go Clear

Limits   Preview/Index   History   Clipboard   Details

Display Abstract ▼ Show 20 ▼ Sort by ▼ Send to ▼

About Entrez

Text Version

All: 1   Review: 0

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Eur J Vasc Endovasc Surg. 2001 Oct;22(4):310-6.   Related Articles, Links

ELSEVIER
FULL-TEXT ARTICLE

The effect of vascular endothelial growth factor on angiogenesis: an experimental study.

Becit N, Ceviz M, Kocak H, Yekeler I, Unlu Y, Celenk C, Akin Y.

Department of Cardiovascular Surgery, 19 Mayis University School of Medicine, Erzurum, Turkey.

OBJECTIVE: to evaluate the effects of exogenous vascular endothelial growth factor (VEGF) on angiogenesis in a rabbit model of persistent hind limb ischaemia. MATERIALS AND METHODS: ischaemia was induced in the hind limbs of 42 New Zealand white rabbits divided into six groups, each of 7 animals. Group 1a and 1b received intramuscular injections of 1 and 2 mg VEGF/day, respectively, into the ischaemic hind limb for 10 days beginning on postoperative 11th day, and group 1c received IM injections of saline only. Group 2a and 2b received similar regimen of VEGF, but administered intra-arterially. Group 2c served as controls. Perfusion of the ischaemic limb was evaluated by thigh blood pressure and thigh circumference at 10, 25 and 40 days following limb ischaemia in all animals and by digital subtraction angiography, perfusion scans, histological examination of capillary density in 2 animals from each group. RESULTS: thigh pressure index and thigh circumference improved significantly in the VEGF treated animals (Groups 1a,b and 2a,b). Collateral formation, as assessed by angiography, scintigraphy and by histological examination, indicated marked formation of collaterals in the VEGF treated animals as compared with the controls. This was most pronounced in groups receiving the highest dose of VEGF. CONCLUSION: these data suggest that VEGF promotes angiogenesis, that the route of administration is unimportant, but that a dose-response relationship is present in this experimental ischaemic hind limb model. Copyright 2001 Harcourt Publishers Limited.

PMID: 11563889 [PubMed - indexed for MEDLINE]

Display Abstract ▼ Show 20 ▼ Sort by ▼ Send to ▼

Write to the Help Desk

19TH
EDITION

Remington:
Practice of

ALFONSO   R   GENNARO

Chairman of the Editorial Board
and Editor

The Science and Pharmacy

1995

Table 1—Rates of Entry of Drugs in CSF and the Degrees of Ionization of Drugs at pH 7.4[7]

Drug/chemical	% binding to plasma protein	pKa[a]	% un-ionized at pH 7.4	Permeability constant (P min⁻¹) ± S.E.
		Drugs mainly ionized at pH 7.4		
5-Sulfosalicylic acid	22	(strong)	0	< 0.0001
N-Methylnicotinamide	<10	(strong)	0	0.0005 ± 0.00006
5-Nitrosalicylic acid	42	2.3	0.001	0.001 ± 0.0001
Salicylic acid	40	3.0	0.004	0.006 ± 0.0004
Mecamylamine	20	11.2	0.016	0.021 ± 0.0016
Quinine	76	8.4	9.09	0.078 ± 0.0061
		Drugs mainly un-ionized at pH 7.4		
Barbital	<2	7.5	55.7	0.026 ± 0.0022
Thiopental	75	7.6	61.3	0.50 ± 0.051
Pentobarbital	40	8.1	83.4	0.17 ± 0.014
Aminopyrine	20	5.0	99.6	0.25 ± 0.020
Aniline	15	4.6	99.8	0.40 ± 0.042
Sulfaguanidine	6	>10.0[b]	>99.8	0.003 ± 0.0002
Antipyrine	8	1.4	>99.9	0.12 ± 0.013
N-Acetyl-4-aminoantipyrine	<3	0.5	>99.9	0.012 ± 0.0010

[a] The dissociation constant of both acids and bases is expressed as the pK_a; the negative logarithm of the acidic dissociation constant.
[b] Sulfaguanidine has a very weakly acidic group ($pK_a > 10$) and two very weakly basic groups (pK_a 2.75 and 0.5). Consequently, the compound ;

for all practical purposes, only the un-ionized form is said to pass through the membrane. This has become known as the *principle of nonionic diffusion.*

This principle is the reason that only the concentrations of the un-ionized form of the barbiturates are plotted in Fig 9.

For the purpose of further illustrating the principle, Table 1 is provided.[7] In the table, the permeability constants for penetration into the cerebral spinal fluid of rats are higher for un-ionized drugs than for ionized ones. The apparent exceptions—barbital, sulfaguanidine and acetylaminoantipyrine—

may be explained by the dipolarity of the un-ionized molecule. With barbital, the two lipophilic ethyl groups are too small to compensate for the considerable dipolarity of the un-ionized barbituric acid ring; also it may be seen that barbital is appreciably ionized, which contributes to the relatively small permeability constant. Sulfaguanidine and acetylaminoantipyrine are both very polar molecules. Mecamylamine also might be considered an exception, since it shows a modest permeability even though strongly ionized; there is no dipolarity in mecamylamine except in the amino group.

Absorption of Drugs

Absorption is the process of movement of a drug from the site of application into the extracellular compartment of the body. Inasmuch as there is a great similarity among the various membranes that a drug may pass through in order to gain access to the extracellular fluid, it might be expected that the particular site of application (or *route*) would make little difference to the successful absorption of the drug. In actual fact, it makes a great deal of difference; many factors, other than the structure and composition of the membrane, determine the ease with which a drug is absorbed. These factors are discussed in the following sections, along with an account of the ways that drug formulations may be manipulated to alter the ability of a drug to be absorbed readily.

Routes of Administration

Drugs may be administered by many different routes. The various routes include oral, rectal, sublingual or buccal, parenteral, inhalation and topical. The choice of a route depends upon both convenience and necessity.

Oral Route—This is obviously the most convenient route for access to the systemic circulation, providing that various factors do not militate against this route. Oral administration does not always give rise to sufficiently high plasma concentrations to be effective; some drugs are absorbed unpredictably or erratically; patients occasionally have an absorption malfunction. Drugs may not be given by mouth to patients with gastrointestinal intolerance, or who are in preparation for anesthesia or who have had gastrointestinal surgery. Oral administration also is precluded in coma.

Rectal Route—Drugs that ordinarily are administered by the oral route usually can be administered by injection or by the alternative *lower enteral* route, through the anal portal

into the rectum or lower intestine. With regard to the latter, *rectal suppositories* or *retention enemas* formerly were used quite frequently, but their popularity has abated somewhat owing to improvements in parenteral preparations. Nevertheless, they continue to be valid and, sometimes, very important ways of administering a drug, especially in pediatrics and geriatrics. In Fig 10[8] the availability of a drug by retention enema may be compared with that by the intravenous and oral route and rectal suppository administration. It is apparent that the retention enema may be a very satisfactory means of administration but that rectal suppositories may be inadequate where rapid absorption and high plasma levels are required. The illustration is not intended to lead the reader to the conclusion that a retention enema always will give more prompt and higher blood levels than the oral route, for converse findings for the same drug have been reported, but, rather, to show that the retention enema may offer a useful substitute for the oral route.

Sublingual or Buccal Route—Even though an adequate plasma concentration eventually may be achievable by the oral route, it may rise much too slowly for use in some situations where a rapid response is desired. In such situations parenteral therapy usually is indicated. However, the patients with angina pectoris may get quite prompt relief from an acute attack by the *sublingual* or *buccal* administration of nitroglycerin, so that parenteral administration may be avoided. When only small amounts of drugs are required to gain access to the blood, the buccal route may be very satisfactory, providing the physicochemical prerequisites for absorption by this route are present in the drug and dosage form. Only a few drugs may be given successfully by this route.

Parenteral Routes—These routes, by definition, include any route other than the oral–gastrointestinal (enteral) route
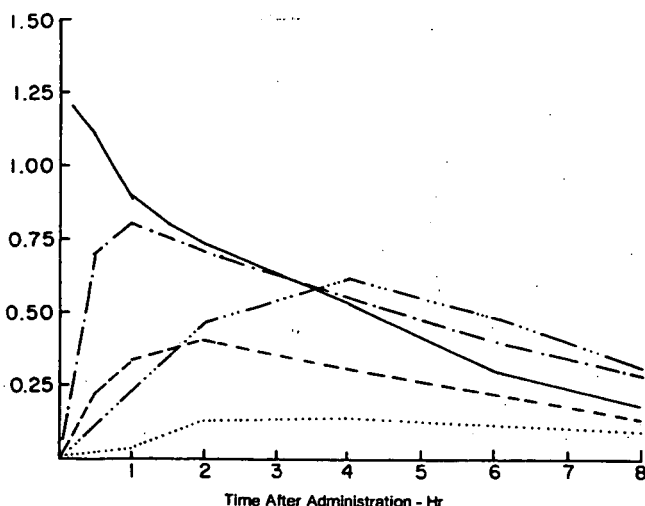
Fig 10. Blood concentration in mg/100 mL of theophylline (ordinate) following administration to humans of aminophylline in the amounts and by the routes indicated. Doses: per 70 kg. Theophylline-ethylenediamine by various routes: —— *intravenous, 0.5 g;* — · — · — *retention enema, 0.5 g;* — ··· — — ··· — *oral tablets-Pl., 0.5 g;* - - - *oral tablets-Pl., 0.3 g;* ···· *rectal suppository, 0.5 g* (courtesy, Truitt *et al*,[8] adapted).

but in common medical usage the term excludes topical administration and includes only various hypodermic routes. Parenteral administration includes the intravenous, intramuscular and subcutaneous routes. Parenteral routes may be employed whenever enteral routes are contraindicated (see above) or inadequate.

The *intravenous* route may be preferred on occasion, even when a drug may be well-absorbed by the oral route. There is no delay imposed by absorption before the administered drug reaches the circulation, and blood levels rise virtually as rapidly as the time necessary to empty the syringe or infusion bottle. Consequently, the intravenous route is the preferred route when an emergency calls for an immediate response.

In addition to the rapid rise in plasma concentration of drug, another advantage of intravenous administration is the greater predictability of the peak plasma concentration, which, with some drugs, can be calculated with a fair degree of precision. Smaller doses generally are required by the intravenous than by other routes, but this usually affords no advantage, inasmuch as the sterile injectable dosage form costs more than enteric preparations and the requirements for medical or paramedical supervision of administration also may add to the cost and inconvenience.

Because of the rapidity with which drug enters the circulation, dangerous side effects to the drug may occur which are often not extant by other routes. The principal untoward effect is a depression of cardiovascular function, which is often called *drug shock*. Consequently, some drugs must be given quite slowly to avoid vasculotoxic concentrations of drug in the plasma. Acute, serious, allergic responses also are more likely to occur by the intravenous route than by other routes.

Many drugs are too irritant to be given by the oral, intramuscular or subcutaneous route and must, of necessity, be given intravenously. However, such drugs also may cause damage to the veins (phlebitis) or, if extravasated, cause necrosis (slough) around the injection site. Consequently, such irritant drugs may be diluted in isotonic solutions of saline, dextrose or other media and given by slow infusion, providing that the slower rate of delivery does not negate the purpose of the administration in emergency situations.

Absorption by the *intramuscular* route is relatively fast and this parenteral route may be used where an immediate effect is not required but a prompt effect is desirable. Intramuscular deposition also may be made of certain repository preparations, rapid absorption not being desired. Absorption from an intramuscular depot is more predictable and uniform than from a subcutaneous site.

Irritation around the injection site is a frequent accompaniment of intramuscular injection, depending upon the drug and other ingredients. Because of the dangers of accidental intravenous injection, medical supervision generally is required. Sterilization is necessary.

In *subcutaneous* administration the drug is injected into the alveolar connective tissue just below the skin. Absorption is slower than by the intramuscular route but, nevertheless, may be prompt with many drugs. Often, however, absorption by this route may be no faster than by the oral route. Therefore, when a fairly prompt response is desired with some drugs, the subcutaneous route may not offer much advantage over the oral route, unless for some reason the drug cannot be given orally.

The slower rate of absorption by the subcutaneous route is usually the reason why the route is chosen, and the drugs given by this route are usually those in which it is desired to spread the action out over a number of hours, in order to avoid either too intense a response, too short a response or frequent injections. Examples of drugs given by this route are insulin and sodium heparin, neither of which is absorbed orally and both of which should be absorbed slowly over many hours. In the treatment of asthma, epinephrine usually is given subcutaneously to avoid the dangers of rapid absorption and consequent dangerous cardiovascular effects. Many repository preparations, including tablets or pellets, are given subcutaneously. As with other parenteral routes, irritation may occur. Sterile preparations also are required. However, medical supervision is not required always and self-administration by this route is customary with certain drugs, such as insulin.

Intradermal injection, in which the drug is injected into, rather than below the dermis, is rarely employed, except in certain diagnostic and test procedures, such as screening for allergic or local irritant responses.

Occasionally, even by the intravenous route, it is not possible, practical or safe to achieve plasma concentrations high enough so that an adequate amount of drug penetrates into special compartments, such as the cerebrospinal fluid, or various cavities, such as the pleural cavity. The brain is especially difficult to penetrate with water-soluble drugs. The name *blood-brain barrier* is applied to the impediment to penetration. When drugs do penetrate, the choroid plexus often secretes them back into the blood very rapidly, so that adequate levels of drugs in the cerebrospinal fluid may be difficult to achieve. Consequently, *intrathecal* or *intraventricular* administration may be indicated.

Body cavities such as the pleural cavity normally are wetted by a small amount of effusate which is in diffusion equilibrium with the blood and, hence, is accessible to drugs. However, infections and inflammations may cause the cavity to fill with serofibrinous exudate which is too large to be in rapid diffusion equilibrium with the blood. *Intracavitary* administration, thus, may be required. It is extremely important that sterile and nonirritating preparations be used for intrathecal or intracavitary administration.

Inhalation Route—Inhalation may be employed for delivering gaseous or volatile substances into the systemic circulation, as with most general anesthetics. Absorption is virtually as rapid as the drug can be delivered into the alveoli of the lungs, since the alveolar and vascular epithelial membranes are quite permeable, blood flow is abundant and there is a very large surface for absorption.

Aerosols of nonvolatile substances also may be administered by inhalation, but the route is used infrequently for delivery into the systemic circulation because of various factors which contribute to erratic or difficult-to-achieve blood levels. Whether or not an aerosol reaches and is retained in pulmonary alveoli depends critically upon particle size. Particles greater than 1 μm in diameter tend to settle in the

bronchioles and bronchi, whereas particles less than 0.5 μm fail to settle and mainly are exhaled. Aerosols are employed mostly when the purpose of administration is an action of the drug upon the respiratory tract itself. An example of a drug commonly given as an aerosol is isoproterenol, which is employed to relax the bronchioles during an asthma attack.

Topical Route—Topical administration is employed to deliver a drug at, or immediately beneath, the point of application. Although occasionally enough drug is absorbed into the systemic circulation to cause systemic effects, absorption is too erratic for the topical route to be used routinely for systemic therapy. However, various transdermal preparations of nitroglycerin and clonidine are employed quite successfully for systemic use. Some investigations with aprotic solvent vehicles such as dimethyl sulfoxide (DMSO) also has generated interest in topical administration for systemic effects. A large number of topical medicaments are applied to the skin, although topical drugs are also applied to the eye, nose and throat, ear, vagina, etc.

In man, percutaneous absorption probably occurs mainly from the surface. Absorption through the hair follicles occurs, but the follicles in man occupy too small a portion of the total integument to be of primary importance. Absorption through sweat and sebaceous glands generally appears to be minor. When the medicament is rubbed on vigorously, the amount of the preparation that is forced into the hair follicles and glands is increased. Rubbing also forces some material through the stratum corneum without molecular dispersion and diffusion through the barrier. Rather large particles of substances such as sulfur have been demonstrated to pass intact through the stratum corneum. When the skin is diseased or abraded, the cutaneous barrier may be disrupted or defective, so that percutaneous absorption may be increased. Since much of a drug that is absorbed through the epidermis diffuses into the circulation without reaching a high concentration in some portions of the dermis, systemic administration may be preferred in lieu of, or in addition to, topical administration.

Factors That Affect Absorption

In addition to the physicochemical properties of drug molecules and biological membranes, various factors affect the rate of absorption and determine, in part, the choice of route of administration.

Concentration—It is self-evident that the concentration, or, more exactly, the thermodynamic activity, of a drug in a drug preparation will have an important bearing upon the rate of absorption, since the rate of diffusion of a drug away from the site of administration is directly proportional to the concentration. Thus, a 2% solution of lidocaine will induce local anesthesia more rapidly than a 0.2% solution. However, drugs administered in solid form are not absorbed necessarily at the maximal rate (see *Physical State of Formulation and Dissolution Rate*, below).

After oral administration the concentration of drugs in the gut is a function of the dose, but the relationship is not necessarily linear. Drugs with a low aqueous solubility (eg, digitoxin) quickly saturate the gastrointestinal fluids, so that the rate of absorption tends to reach a limit as the dose is increased. The peptizing and solubilizing effects of bile and other constituents of the gastrointestinal contents assist in increasing the rate of absorption but are in themselves somewhat erratic. Furthermore, many drugs affect the rates of gastric, biliary and small intestinal secretion, which causes further deviations from a linear relationship between concentration and dose.

Drugs that are administered subcutaneously or intramuscularly also may not always show a direct linear relationship between the rate of absorption and the concentration of drug in the applied solution, because osmotic effects may cause dilution or concentration of the drug, if the movement of water or electrolytes is different from that of the drug. Whenever possible, drugs for hypodermic injection are prepared as isotonic solutions. Some drugs affect the local blood flow and capillary permeability, so that at the site of injection there may be a complex relationship of concentration achieved to the concentration administered.

Physical State of Formulation and Dissolution Rate—The rate of absorption of a drug may be affected greatly by the rate at which the drug is made available to the biological fluid at the site of administration. The intrinsic physicochemical properties, such as solubility and the thermodynamics of dissolution, are only some of the factors which affect the rate of dissolution of a drug from a solid form. Other factors include not only the unavoidable interactions among the various ingredients in a given formulation but also deliberate interventions to facilitate dispersion (eg, comminution, Chapter 83 and dissolution, Chapter 34) or retard it (eg, coatings, Chapter 93 and slow-release formulations, Chapter 94). There also are factors that affect the rate of delivery from liquid forms. For example, a drug in a highly viscous vehicle is absorbed more slowly from the vehicle than a drug in a vehicle of low viscosity; in oil-in-water emulsions, the rate depends upon the partition coefficient. These manipulations are the subject of biopharmaceutics (see Chapter 94).

Area of Absorbing Surface—The area of absorbing surface is an important determinant of the rate of absorption. To the extent that the therapist must work with the absorbing surfaces available in the body, the absorbing surface is not subject to manipulation. However, the extent to which the existing surfaces may be used is subject to variation. In those rare instances in which percutaneous absorption is intended for systemic administration, the entire skin surface is available.

Subsequent to subcutaneous or intramuscular injections, the site of application may be massaged in order to spread the injected fluid from a compact mass to a well-dispersed deposit. Alternatively, the dose may be divided into multiple small injections, although this recourse is generally undesirable.

The different areas for absorption afforded by the various routes account, in part, for differences in the rates of absorption by those routes. The large alveolar surface of the lungs allows for extremely rapid absorption of gases, vapors and properly aerosolized solutions; with some drugs the rate of absorption may be nearly as fast as intravenous injection. In the gut the small intestine is the site of the fastest, and hence most, absorption because of the small lumen and highly developed villi and microvilli; the stomach has a relatively small surface area, so that even most weak acids are absorbed predominately in the small intestine despite a pH partition factor that should favor absorption from the stomach (see *The pH Partition Principle*, page 715).

Vascularity and Blood Flow—Although the thermal velocity of a freely diffusible average drug molecule is on the order of meters per second, in solution the rate at which it will diffuse away from a reference point will be much slower. Collisions with water and/or other molecules, which cause a random motion, and the forces of attraction between the drug and water or other molecules slow the net mean velocity.

The time taken to traverse a given distance is a function of the square of the distance; on the average it would take about 0.01 second for a net outward movement of 1 μm, 1 second for 10 μm, 100 second for 100 μm, etc. In a highly vascular tissue, such as skeletal muscle, in which there may be more than 1000 capillaries/mm^2 of cross section, a drug molecule would not have to travel more than a few microns, hence, less than a second on the average, to reach a capillary from a point of extravascular injection.

Once the drug reaches the blood, diffusion is not important to transport and the rate of blood flow determines the movement. The velocity of blood flow in a capillary is about 1 mm/sec, which is 100 times faster than the mean net velocity of drug molecules 1 mm away from their injection site. The velocity of blood flow is even faster in the larger vessels. Overall, less than a minute is required to distribute drug molecules from the capillaries at the injection site to the rest of the body.

The Biology of Leptin: A Review

Karen L. Houseknecht*, Clifton A. Baile†, Robert L. Matteri‡,
and Michael E. Spurlock§

*Department of Animal Sciences, Purdue University, West Lafayette, IN 47907
and Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202;
†Departments of Animal Science and Food and Nutrition, University of Georgia, Athens 30602;
‡Animal Physiology Research Unit, Agricultural Research Service, USDA,
Columbia, MO 65201; and §Purina Mills, Inc., St. Louis, MO 63144

ABSTRACT: Leptin, a 16-kDa protein secreted from white adipocytes, has been implicated in the regulation of food intake, energy expenditure, and whole-body energy balance in rodents and humans. The gene encoding leptin was identified by positional cloning and is the mutation leading to the profound obese phenotype of the *ob/ob* mouse. Exogenous administration of leptin to *ob/ob* mice leads to a significant improvement in reproductive and endocrine status as well as reduced food intake and weight loss. The expression and secretion of leptin is highly correlated with body fat mass and adipocyte size. Cortisol and insulin are potent stimulators of leptin expression, and expression is attenuated by β-adrenergic agonists, cAMP, and thiazolidinediones. The role of other hormones and growth factors in the regulation of leptin expression and secretion is emerging. Leptin circulates specifically bound to proteins in serum, which may regulate its half-life and biological activity. Isoforms of the leptin receptor, members of the interleukin-6 cytokine family of receptors, are found in multiple tissues, including the brain. Many of leptin's effects on food intake and energy expenditure are thought to be mediated centrally via neurotransmitters such as neuropeptide Y. Multiple peripheral effects of leptin have also been recently described, including the regulation of insulin secretion by pancreatic β cells and regulation of insulin action and energy metabolism in adipocytes and skeletal muscle. Leptin is thought to be a metabolic signal that regulates nutritional status effects on reproductive function. Leptin also plays a major role in hematopoiesis and in the anorexia accompanying an acute cytokine challenge. The profound effects of leptin on regulating body energy balance make it a prime candidate for drug therapies for humans and animals.

Introduction

Obesity is a major health issue in much of the human population. In the United States, it is estimated that over 30% of the population is overweight by at least 20%, and this proportion is increasing. A conservative estimate over 10 yr ago of the total economic costs of obesity in the United States was $39.3 billion, considering all of the associated diseases; more recent estimates are much higher (Colditz, 1992). It is also estimated that at least $30 billion is spent to treat obesity in the United States annually (Gura, 1997). Most attempts to treat obesity to date, except for the several types of surgical removal of the tissue, have failed to result in a sustained reduction of obesity. An example of the sensitivity of the physiology of weight maintenance is that the average woman may gain 11 kg between the ages of 25 and 65, and this is the result of only 350 mg of excess daily food on the average. This is in the face of more than 18 t of food intake over the 40 yr, or an error of less than .03%.

Obesity per se is not a major concern for animal agriculture. However, altering body composition by repartitioning of nutrients to favor lean protein accretion and improve productive efficiency are major goals for animal scientists. Furthermore, regulation of feed intake and whole-body energy balance in livestock species is important for optimizing animal growth, reproduction, lactation, and overall health and well-being. Thus, understanding the basic mechanisms that regulate adiposity, feed intake, and energy metabolism in livestock may lead to new technologies that will further enhance animal performance and health.

Table 1. Single-gene obesity mutations in rodents

Name	Allele	Gene Product	Inheritance	Degree/ onset	Chromosome
Mice					
Agouti	Ay	AGOUTI protein	Dominant	Moderate/adult	2
Obese	ob	OB protein (leptin)	Recessive	Extreme/early	6
Diabetes	db	OB-R receptor	Recessive	Extreme/early	4
Fat	fat	Carboxypeptidase E	Recessive	Moderate/adult	8
Tubby	tub	TUB protein	Recessive	Moderate/adult	7
Rats					
Zucker	fa/fa	OB-R receptor			5
Corpulent	La/nf/f	OB-R receptor			5

History and Features of *ob/ob* Mice

Research of human obesity and its treatment has included extensive use of animal models. One of the early models identified is the *ob/ob* mouse, discovered in 1950 (Ingalls et al., 1950). Animal caretakers at the Jackson Laboratories discovered this mouse on the C57BL/6J background. Over the years, these mice have been the subject of many *Science* and *Nature* "breakthrough" publications.

The *ob/ob* mice have a recessive genetic obesity that results in sterile adult mice with over 50% fat. A close relative, *db/db*, was later discovered; these mice arose on the C57BL/KSJ background. These mice were similarly obese but also were hyperglycemic (Hummel et al., 1966). Coleman, of the Jackson Laboratories, reported seminal studies using parabiosis (cross-circulation) of these mice (Coleman, 1973). Research with parabiotic pairs of the *ob/ob* and *db/db* mice showed a decrease in food intake and body weight in the *ob/ob* pair-mates and a retention of increased food intake and weight in their *db/db* pair-mates. Coleman concluded that *ob/ob* mice fail to make a circulating factor from adipose tissue but their brains can respond to it and reduce food intake, whereas *db/db* mice make the circulating factor in their adipose tissue but their brains cannot respond to it. It was not until 1995 that studies were possible to verify these hypotheses.

Discovery of the Ob Gene

Even though many observations of the metabolism and physiology of the *ob/ob* mice were reported, the real genetic defect was impossible to elucidate until the powerful tools of biotechnology were available. A team led by Jeffrey Friedman at Rockefeller University published the specific gene defect of *ob/ob* mice in December 1994 after an 8-yr search (Zhang et al., 1994). Three papers in 1995 clearly showed that the ob protein, leptin, eliminated the obesity of the *ob/ob* mice (Halaas et al., 1995; Pelleymounter et al., 1995; Rentsch et al., 1995), and these have led to an estimated 250 publications in 1996 and nearly another 250 publications in the first half of 1997 on leptin biology and chemistry. Also associated with this discovery has been a huge influx of industrial investment into research and applications for novel treatments of obesity.

The *ob/ob* mouse produces no leptin, due to the gene mutation. The genetic basis of other obesities in rodents have now been reported and are summarized in Table 1. It is interesting that none of these has led to an explanation of any prevalent form of human obesity. In 1997, a very infrequent form of hunger and obesity in humans was reported in two patients; it was confirmed to be the result of a lack of leptin production by their adipocytes (Montague et al., 1997). It has been proposed that a composite of genes is likely to be responsible for most human obesities, and therefore much more research will be required to make major strides in the understanding of this human condition.

The leptin gene has been cloned in pigs (Bidwell et al., 1997), and the cloning of leptin genes in other livestock species will likely be reported in the near future. Due to the lack of published data in farm animals, the importance of leptin as a genetic marker for animal growth, reproductive, and lactational performance remains to be determined.

Discovery and Function of Leptin Receptors

Tartaglia and coworkers (Tartaglia et al., 1995) reported the cloning of the leptin receptor in December 1995. A high-affinity leptin receptor was cloned from mouse choroid plexus (Tartaglia et al., 1995) and was genetically mapped to the same interval of mouse chromosome 4 that contains the *db* locus (Tartaglia et al., 1995). In the *db/db* mouse, the mRNA for the long form of the receptor is abnormal and yields a receptor with a truncated intracellular domain that is unable to appropriately signal (Chen et al., 1996b; Chua et al., 1996; Ghilardi et al., 1996; Lee et al., 1996; Vaisse et al., 1996), thus confirming the prediction made by Coleman based on parabiosis studies.

Several splice variants of the single gene exist, including the "long" form of the receptor that contains a 302 amino acid intracellular domain. The long form of the receptor is expressed in various regions of the brain and is thought to be responsible for the central

actions of leptin (Tartaglia et al., 1995; Glaum et al., 1996; Mercer et al., 1996b; Elmquist et al., 1997). The helical structure of leptin implied that the leptin receptor would be similar in structure and function to the helical cytokine receptors. This expectation was confirmed when the receptor was determined to be similar to the gp130 signal transduction arm of class I cytokine receptor family members, interleukin (IL)-6, granulocyte colony stimulating factor (G-CSF), and leukemia inhibitory factor (LIF; Tartaglia et al., 1995). The extracellular domain of the long (OB-RL) and short (OB-RS) forms includes two cytokine domains, each containing a single copy of the characteristic Trp-Ser-X-Trp-Ser motif and a fibronectin type III domain (White and Tartaglia, 1996).

The similarity of the leptin receptor to the class I cytokine receptor family has implications for signal transduction mechanisms. Class I cytokine receptor members typically lack intrinsic tyrosine kinase activity and are activated by formation of homo- or heterodimers (Watowich et al., 1996). Leptin receptors have been reported to form homodimers (Nakashima et al., 1997; White et al., 1997). Briefly, following ligand-receptor binding and receptor aggregation, phosphorylation events ultimately result in activation of Janus kinases (JAK). The JAK then phosphorylate specific receptor tyrosine residues that provide docking sites for members of the signal transducers and activators of transcription (STAT) family. Phosphorylation of these transcription factors by JAK is followed by their dimerization and translocation to the nucleus for regulatory purposes.

Leptin signaling via the JAK-STAT pathway has been reasonably well documented and is associated largely with the OB-RL isoform. In mice, administration of recombinant leptin causes activation of STAT3 in the hypothalamus of wild-type and *ob/ob* mice (Vaisse et al., 1996). The STAT3 and STAT5 are activated in COS cells following ligand binding to OB-RL, but results for STAT1 and STAT6 were equivocal (Ghilardi and Skoda, 1997). However, Rosenblum et al. (1996) reported formation of STAT1, STAT3, and mixed STAT1:STAT3 dimers in cells transfected with OB-RL and treated with leptin. Tyrosine phosphorylation of STAT1 has also been demonstrated in a human renal adenocarcinoma cell line after treatment with leptin (Takahashi et al., 1996).

Signal transduction by members of the class I cytokine receptor family is not limited to the JAK-STAT system. Some of these receptors are linked to mitogen-activated protein kinase (MAPK) or phosphotidyl inositol-3 (PI-3) kinase pathways (Watowich et al., 1996). It is yet to be determined whether ligand binding to one or more of the leptin receptor isoforms activates signaling systems other than the JAK-STAT pathway and which biochemical processes are regulated through them.

Even though leptin is exclusively produced and secreted by adipocytes (Kline et al., 1997) and placenta (Gong et al., 1996), leptin receptors are found in most tissues. The long form is especially prevalent in the hypothalamus (arcuate, lateral, ventromedial, and dorsomedial nuclei) and not present in most other tissues (Mercer et al., 1996b; Schwartz et al., 1996c), and the short forms are more ubiquitously expressed. It has been proposed that some of the receptor forms are involved in the transport of leptin in blood and its crossing of the blood-brain barrier (Devos et al., 1996).

Roles of Leptin in Energy Balance Regulation

In the fed, steady-state (zero energy balance), leptin expression and secretion reflect body fat mass in rodents and humans (Frederich et al., 1995a; Maffei et al., 1995; Considine et al., 1996) and are highly correlated with adipocyte size in lean and obese mice (Houseknecht et al., 1996a,b). This correlation with fat mass is drastically altered, however, with changes in energy balance. Food deprivation (12 to 48 h) results in a rapid and drastic fall in leptin gene expression (Cusin et al., 1995; Frederich et al., 1995b; Trayhurn et al., 1995; Kolaczynski et al., 1996a). However, more subtle changes in energy balance have profound effects on leptin expression as well. As little as a 10% reduction in body weight in obese human subjects results in a 53% reduction in serum leptin (Considine et al., 1996), and a 10% increase in body weight causes a 300% increase in serum leptin (Kolaczynski et al., 1996b). Thus, leptin not only functions as an "adipostat" to signal the status of body energy stores to the brain (and perhaps other tissues), but also functions as a sensor of energy balance.

Leptin treatment of animals has been shown to cause a dose-dependent decrease in food intake, loss of body weight, loss of fat depots, and an increase in energy metabolism (Pelleymounter et al., 1995; Levin et al., 1996). Leptin treatments can be used to eliminate all visible fat in rodents (Chen et al., 1996a). The body weight and fat losses are unusual following leptin treatment in that the loss of body weight and fat depots are not repleted for several weeks after the termination of the leptin treatment (Chen et al., 1996a; Azain et al., 1997). Therefore, leptin not only causes reduced food intake, but the potential body weight losses are enhanced due to an increased metabolic rate. This is in contrast to the reduced metabolic rate associated with limited feeding. These effects are observed independently of mode of administration (central vs systemic), although higher doses are required with systemic treatment (Campfield et al., 1995).

Leptin is known to act centrally to inhibit the effects of neuropeptide Y (NPY), apparently by inhibiting its synthesis in the arcuate nucleus of the hypothalamus (Cusin et al., 1996; Erickson et al.,

1996b). There are likely many unknown components of the leptin axis to be discovered to explain its many functions, but it is likely that some of the actions are mediated by the beta-adrenergic enervation of adipose tissue (Collins et al., 1996) and by the uncoupling proteins I and II (Zhou et al., 1997).

Endocrine Regulation of Leptin Gene Expression and Secretion

Coordinated alterations in leptin gene expression with changing metabolic status suggest hormonal or metabolite control of leptin expression. A prime candidate for such regulation is insulin. Insulin plays a chronic (hours) role in the regulation of leptin levels. Hyperinsulinemia increases leptin levels following 3 to 5 h in rodents and humans (Cusin et al., 1995; Saladin et al., 1995; Utriainen et al., 1996; Vidal et al., 1996), and in vitro exposure of rat adipocytes to insulin (12 to 48 h) increased leptin mRNA levels (Slieker et al., 1996). Leptin concentrations in blood are diurnally regulated in rodents and humans (Saladin et al., 1995; Sinha et al., 1996a), with levels peaking at night. In rodents, the peak coincides with the initiation of eating behavior, and the elevation is inhibited by fasting (Frederich et al., 1995b; MacDougald et al., 1995; Saladin et al., 1995) and can be reinstated following a meal or a single injection of insulin (Saladin et al., 1995). In humans, the nocturnal rise in leptin levels does not coincide with feeding (Sinha et al., 1996a), suggesting differential regulation in these two species. We (Houseknecht et al., 1996a) and others (Slieker et al., 1996) have observed no acute (minutes) regulation of leptin secretion by insulin and have found no intracellular storage pool for leptin that could be rapidly mobilized in response to secretagogues such as insulin (Houseknecht et al., 1996a). It should be noted, however, that leptin concentrations in human plasma are pulsatile in nature (Licinio et al., 1997), suggesting some yet to be discovered control of leptin secretion and(or) clearance.

Glucocorticoids are potent regulators of leptin expression. The in vivo administration (De Vos et al., 1995) and in vitro incubation of adipocytes (Slieker et al., 1996; Wabitsch et al., 1996) with various glucocorticoids causes an up-regulation of leptin expression. Recent evidence indicates that leptin and cortisol interact in a negative feedback loop; leptin directly inhibits cortisol synthesis by adrenal cells (Bornstein et al., 1997).

Leptin expression and secretion by adipocytes are down-regulated by adrenergic stimulation, as indicated in studies using β_3-adrenergic agonists, cold exposure, or dbcAMP (Gettys et al., 1996; Mantzoros et al., 1996; Slieker et al., 1996; Trayhurn et al., 1996). Leptin has been reported to regulate growth hormone secretion (Carro et al., 1997); however,

chronic incubation of isolated adipocytes with either growth hormone or IGF-I had no effect on leptin expression and secretion (Hardie et al., 1996).

The Leptin Promoter: C/EBPα, PPARγ, and Leptin Expression

Initial studies of the leptin promoter have revealed functional binding sites for C/EBPα (CCAAT/enhancer binding protein α) in the region −58 to −42 relative to the transcriptional start site (He et al., 1995; MacDougald et al., 1995; Hwang et al., 1996). The C/EBPα is expressed in multiple cell types, functions as a transcriptional activator of certain adipocyte genes (Christy et al., 1989; Herrera et al., 1989), and plays a role in terminal adipocyte differentiation (Umek et al., 1991; Freytag and Geddes, 1992).

Transcriptional regulation of the leptin gene also seems to be controlled by PPARγ. The PPARγ1 and 2 are members of the family of orphan nuclear receptors that function as trans-activators of fat-specific genes such as aP2, and thus are dominant activators of fat cell differentiation (Tontonoz et al., 1994; Schoonjans et al., 1996a; Schoonjans et al., 1996b). Thiazolidinediones (**TZD**), pharmacological ligands for the PPARγ (Lehmann et al., 1995; Saltiel and Olefsky, 1996), act to down-regulate leptin mRNA abundance in adipocytes. These data are consistent for a role of PPARγ in regulating the leptin promoter (Kallen and Lazar, 1996; Nolan et al., 1996; Zhang et al., 1996). Hollenberg et al. (1997) recently reported the presence of a canonical DR+1 PPARγ binding site located between −3,951 and −3,939 of the mouse 5′-flanking sequence of the leptin gene. Furthermore, these authors report that PPARγ2 mediates down-regulation of the leptin promoter by inhibiting C/EBP α-mediated transactivation (Hollenberg et al., 1997).

Mechanisms of Leptin Action: Central vs Peripheral Effects

The lipostatic theory of body weight maintenance, which was developed from parabiosis data (Kennedy, 1953; Hervey, 1958; Hausberger, 1959; Coleman and Hummel, 1969), proposed a fat-secreted "factor" that reports the status of body energy stores to the brain and thus regulates feeding behavior and body fat mass. Studies examining the effects of exogenous leptin administration to ob/ob mice have led to the development of a deceivingly simple model of leptin-initiated energy balance regulation that is reminiscent of the lipostatic theory (Figure 1).

Leptin is synthesized and secreted from white adipocytes into blood and is transported into the brain via a saturable system (Banks et al., 1996), where it acts to cause the release or inhibition of factors that ultimately result in a reduction in food intake, an
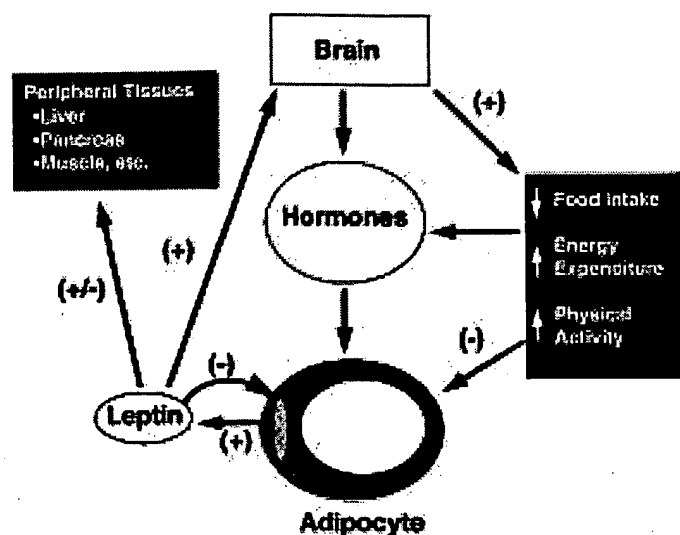
Figure 1. Schematic illustration of leptin secretion and action. Leptin is secreted from white adipocytes into the circulation. Leptin travels to the brain, where it acts to cause the stimulation or inhibition of release of neu-rotransmitters such as neuropeptide Y (NPY), which act to inhibit food intake and stimulate thermogenesis and physical activity in rodents. Such functions result in a reduction in adipose mass or the stimulation of some other endocrine or auto/paracrine signal that inhibits leptin synthesis and secretion by adipocytes. Leptin may also directly affect the metabolism and function of peripheral tissues such as liver and skeletal muscle as well as adipocytes. (+), stimulation; (−) inhibit; (+/−) stimulate and(or) inhibit; ↑, increase; ↓, decrease.

increase in energy expenditure (due at least in part by brown adipose tissue thermogenesis in rodents), and increased physical activity. Additionally, leptin (and perhaps other factors) acts in a negative feedback loop to inhibit further expression of the leptin gene.

Leptin treatment of ob/ob mice causes a rapid reduction in food intake, increased thermogenesis, increased physical activity, and improved glycemia and insulinemia, which occur prior to weight loss (Campfield et al., 1995; Halaas et al., 1995; Pelley-mounter et al., 1995; Stephens et al., 1995; Weigle et al., 1995; Schwartz et al., 1996a).

Many of leptin's effects on the control of food intake and energy expenditure are thought to be mediated centrally. Intense investigation is underway to deline-ate the targets of leptin action in the brain as well as their downstream effectors. Neuropeptide Y has emerged as a major target of leptin action. The NPY is a potent stimulator of food intake and inhibitor of brown fat thermogenesis (Billington et al., 1991) and increases plasma insulin and corticosteroid levels (Billington et al., 1991; Dryden and Williams, 1996). The expression of NYP in the hypothalamus is increased in many obese rodent models (including ob/ob) and with fasting in rats (Marks et al., 1992;

Wilding et al., 1993). Leptin treatment lowers NPY levels in ob/ob mice (Stephens et al., 1995; Schwartz et al., 1996a), and this occurs before any change in body weight. Additionally, leptin treatment directly suppressed NPY release from perfused rat hypothalami from normal animals (Stephens et al., 1995). The central role of NPY in leptin action is emphasized by studies in which NPY was knocked out in ob/ob mice (Erickson et al., 1996b). Absence of NPY attenuated, but did not completely normalize, all aspects of the obesity phenotype in ob/ob mice. These data indicate that NPY is not the only neuroendocrine target of leptin, a fact further supported by data showing that normal mice lacking NPY control their food intake and body weight normally and have a normal response to leptin (Erickson et al., 1996a).

Mechanisms of Leptin Action: Peripheral Effects

Although significant evidence exists suggesting that leptin effects are mediated centrally via neuropeptides such as NPY, there is mounting evidence that leptin may act peripherally as well (Figure 2). Leptin receptors are found outside the central nervous system (Tartaglia et al., 1995); however, in many tissues the short form of the receptor predominates (Tartaglia et al., 1995), and it is not yet clear how effectively the truncated forms of the leptin receptor signal. Leptin has been implicated in causing peripheral insulin resistance by attenuating insulin action (and perhaps insulin signaling) in various insulin-responsive cell types. Exposure of HepG2 cells (Cohen et al., 1996) or rat1 fibroblasts (Kroder et al., 1996) to physiological concentrations of leptin in vitro for minutes, hours, or days results in attenuation of insulin-stimulated phosphorylation of IRS-1 (Cohen et al. 1996; Kroder et al., 1996) and, surprisingly, increased PI3 kinase activity (Cohen et al., 1996). In contrast, glucose uptake and glycogen synthesis were increased with leptin exposure of the C2/C12 muscle cell-line; PI3-kinase was implicated in the leptin effects (Berti et al., 1997). A potentially troubling caveat of studies utilizing cultured cells is that the leptin receptor subtypes present in these cell models may not represent those found in primary liver, muscle, or adipose cells. Recently, an elegant study using primary rat adipocytes (Muller et al., 1997) revealed, for the first time in primary insulin-responsive cells, an attenuation of insulin-stimulated glucose metabolism with in vitro exposure to leptin. Addition-ally, leptin was recently reported to alter lipid partitioning, but not insulin-stimulated glucose metabolism, in isolated mouse skeletal muscle (Muoio et al., 1997).

Leptin may also elicit its effects on peripheral insulin resistance by affecting insulin secretion. Lep-tin receptors have been found on pancreatic β-cells (Kieffer et al., 1996), and leptin has been reported to
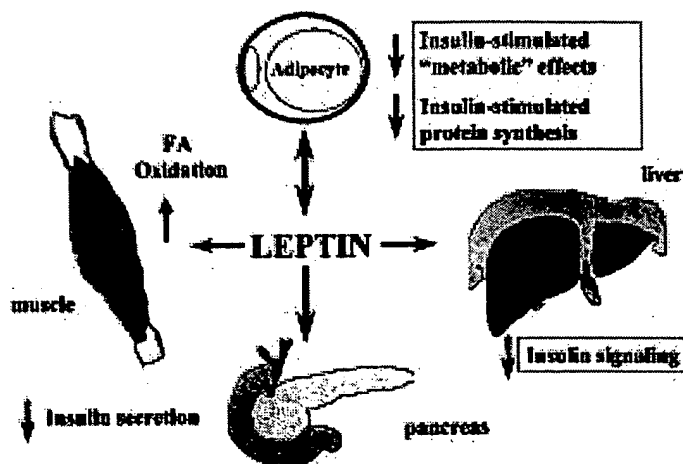
Figure 2. Regulation of peripheral tissue metabolism and function by leptin. Leptin receptors are present on peripheral tissues, and leptin has been shown to directly affect peripheral tissue metabolism and function. Leptin treatment of adipocytes reduces insulin stimulation of carbohydrate and lipid metabolism as well as insulin stimulation of protein synthesis. Leptin treatment of isolated mouse muscle causes an increase in fatty acid oxidation with no change in carbohydrate metabolism. Leptin exposure of pancreatic islets causes a reduction in insulin secretion, and leptin inhibits insulin signaling pathways in the HepG2 liver cell line. ↑, increase, ↓, decrease.

directly inhibit β-cell secretion of insulin by altering ion channel function (Emilsson et al., 1997; Kieffer et al., 1997).

A newly described peripheral action of leptin is to regulate the secretion of cortisol, a powerful stimulator of leptin expression. At concentrations in the range found in obese humans (100 ng/mL), leptin reduced cortisol secretion 52% in isolated adrenocortical cells (Bornstein et al., 1997).

Leptin Resistance

The discovery that mutations in the leptin and leptin receptor genes caused severe obesity in rodents led to the hypothesis that a similar phenomenon was true for humans. In fact, with the exception of the ob/ob mouse and two children from one family of Pakistani origin (Montague et al., 1997), all models of rodent and human obesity studied are characterized not by leptin deficiency, but by hyperleptinemia (see reviews, Caro et al., 1996b; Spiegelman and Flier, 1996). This has led to the concept of leptin resistance (Caro et al., 1996b; Spiegelman and Flier, 1996). Figure 3 illustrates possible molecular scenarios that could lead to leptin resistance.

The most obvious target for a defect in leptin action is the leptin receptor. Defects in receptor expression or proximal signaling events in the brain could result in

severe leptin resistance as observed in the db/db mouse. Additionally, downstream targets and effectors (many yet undefined) of leptin may be defective in certain forms of obesity.

Circulatory "defects" may also result in leptin resistance. The molecular form in which hormones circulate in blood can have a major impact on their biological activity. Many members of the cytokine family circulate bound to proteins in serum, and these binding proteins may play important roles in regulating hormone clearance rates, increasing or decreasing biological activity of the ligand, and(or) providing hormone responsiveness to unresponsive cells (Heaney and Golde, 1993; Bonner and Brody, 1995). Houseknecht et al. (1996c) reported that leptin circulates specifically bound to at least three proteins in mouse serum and, in addition to Sinha et al. (1996b) and Diamond et al. (1997), provided evidence of leptin binding proteins in humans. Figure 4 illustrates a ligand blot showing specific binding of [125I]leptin to proteins in mouse and human serum. Sinha et al. (1996b) and Houseknecht et al. (1996c) found that in lean mice and humans, the majority of leptin circulates in the bound form, and that the proportion of free leptin is positively correlated with increasing obesity and body-mass index (BMI), indicating that leptin binding proteins are saturated with obesity. The precise identity of these proteins is currently unknown, as are the mechanism(s) that regulate their expression and their interaction with the leptin molecule. The leptin-binding protein interactions involve sulfhydryl bonds (Houseknecht et al., 1996c). Many cytokines circulate bound to soluble forms of their receptors (Heaney and Golde, 1993; Bonner and Brody, 1995). The leptin receptor is predicted to have multiple splice variants, including a proposed soluble receptor with predicted molecular weight of 85 kDa. In mice, we have reported specific leptin binding to a protein of approximately 85 kDa (Houseknecht et al., 1996c, see Figure 4). In humans, Caro's group (Sinha et al., 1996b) reported that only approximately 10% of leptin could be immunoprecipitated from serum using a leptin receptor antibody, suggesting that a soluble leptin receptor plays only a minor role in the human. It has been shown that leptin is transported into the brain by a saturable system (Banks et al., 1996; Caro et al., 1996a; Schwartz et al., 1996b), and that the efficiency of leptin transport is reduced in obese patients. It is possible that the bound form of leptin is the biologically active form, and(or) is necessary for transport across the blood-brain barrier. It is likely that the various serum leptin binding proteins affect leptin bioactivity and may be important in the development and(or) manifestation of leptin resistance.

A final possibility is that leptin resistance is not due to a pathological defect in leptin bioactivity, but may simply reflect the limitations of the leptin "system" to regulate food intake and body fat stores. Spiegelman and Flier (1996) proposed that the teleological role of leptin is not to avoid obesity, but to
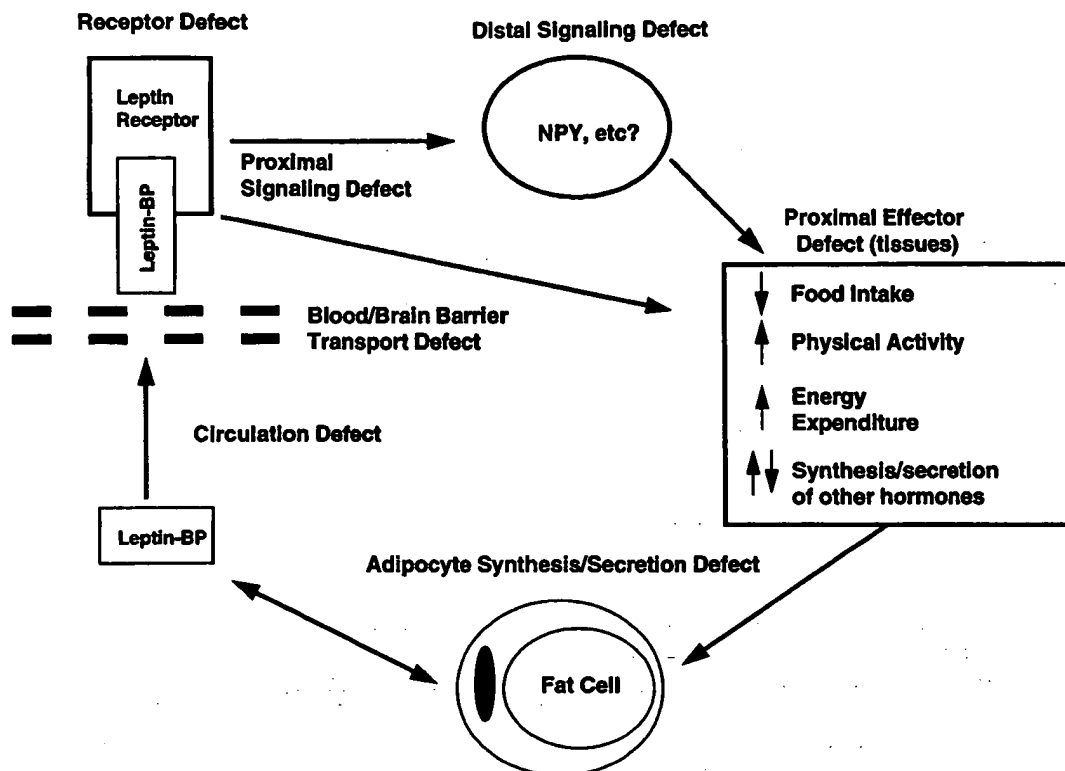
Receptor Defect

Distal Signaling Defect

Leptin Receptor

Leptin-BP

Proximal Signaling Defect

NPY, etc?

Proximal Effector Defect (tissues)

Blood/Brain Barrier Transport Defect

↓ Food Intake

↑ Physical Activity

↑ Energy Expenditure

↑↓ Synthesis/secretion of other hormones

Circulation Defect

Leptin-BP

Adipocyte Synthesis/Secretion Defect

Fat Cell

Figure 3. Potential mechanisms of leptin resistance. Leptin resistance may be due to a defect in leptin binding protein abundance or profile, or in the transport of leptin across the blood-brain barrier. Leptin resistance may also be due to a leptin receptor signaling defect or a defect at the level of effector tissues. Finally, leptin resistance could be due to a synthesis/secretion defect occurring at the level of the adipocyte. NPY, neuropeptide Y; Leptin-BP, leptin binding proteins; ↑, increase, ↓, decrease.

prevent death by starvation. Leptin could be a key regulator of survival during cycles of "feast and famine". This hypothesis is supported by the finding that exogenous leptin administration is able to at least partially overcome the neuroendocrine adaptations to starvation in mice (Ahima et al., 1996). Further research is necessary to support/refute or refine the leptin resistance hypothesis.

Role of Leptin in the Regulation of Reproduction

The body of literature on the role of leptin in the control of reproduction is rapidly developing. At this time, the majority of information in this area has been obtained from research with human subjects and laboratory rodents. Information on domestic livestock is emerging but is limited due to the lack of sufficient quantities of recombinant leptin for whole-animal experiments and the lack of validated assays that can be used to monitor serum leptin secretion.

Nutrition-Reproduction Interactions

The importance of adequate nutritional intake in maintaining reproductive function is well-established (Asdell, 1949; Short and Bellows, 1971; Keane, 1975;

Kirkwood and Aherne, 1985; Armstrong and Britt, 1987). Inadequate nutrition delays or prevents the onset of puberty (Foster and Olster, 1985; Bronson, 1986; Aubert and Sizonenko, 1996) and interferes with normal cyclicity (Howland, 1971; Vigersky et al., 1977; Armstrong and Britt, 1987). In males, under-nutrition is accompanied by hypogonadism and infertility (Brown, 1994). A consistent observation across species is that undernutrition results in decreased gonadotropin secretion (Kennedy and Mitra, 1963; Howland, 1971; Vigersky et al., 1977; Landefeld et al., 1989; Cameron et al., 1993).

The mechanisms responsible for communicating nutritional status to the reproductive system have been sought for many years. A commonly held belief at one time was that the amount of body fat was a controlling factor in the onset of puberty and the maintenance of adult reproduction. More recent research has shown that metabolic and(or) nutritionally induced changes in reproductive function can occur without changes in body fat (Pettigrew and Tokach, 1991; Beltranena et al., 1993). A variety of hormones may act as possible signals of nutritional status to the reproductive system (Pettigrew and Tokach, 1991). Metabolites can exert potent effects on endocrine systems (Widmaier, 1992) and have been implicated in the control of gonadotropin secretion
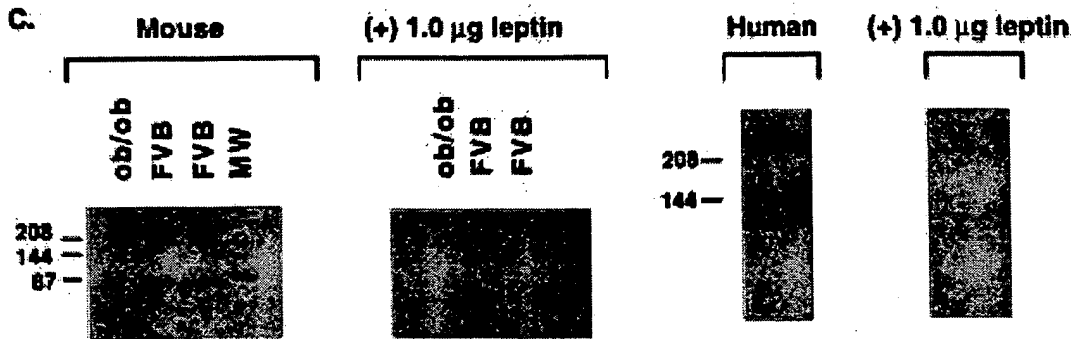
Figure 4. Radioligand binding of [^{125}I]leptin to proteins in serum of mice and humans. Sera from normal FVB or *ob/ob* mice and from non-obese women were electrophoresed in the presence of 4% SDS without reducing agents and blotted with [^{125}I]leptin (± 1.0 μg recombinant leptin). Adapted from Houseknecht et al. (1996c) with permission.

(Pettigrew and Tokach, 1991). To date, the mechanism of nutritional signaling to the reproductive system has not been clearly elucidated.

The relatively recent discovery of leptin (Zhang et al., 1994) has generated considerable excitement in the area of reproductive biology. Many believe that this hormone could be the long-sought indicator of nutritional status that allows reproductive processes to proceed. Leptin is produced in adipose tissue, which actively responds to nutritional and metabolic changes. The production of leptin increases with feeding and body fat content (Hamann and Matthaei, 1996). Leptin receptors are found in the ventromedial and arcuate regions of the hypothalamus and are thus positioned anatomically in regions associated with the control of appetite and reproductive neuroendocrine function (Dyer et al., 1997). Thus, leptin could provide an accurate, circulating signal of nutritional status.

Genetic and Nutritionally Induced Leptin Deficiency

Much of the evidence for the role of leptin as a reproductive hormone has been derived from *ob/ob* mice, which do not produce a functional leptin protein (Hamann and Matthaei, 1996). An *ob/ob* female mouse is sterile and remains essentially in a constant prepubertal state. Ovarian and uterine weights, sex steroid concentrations, and pituitary gonadotropin secretion are depressed in these animals (Barash et al., 1996). Administration of recombinant leptin to *ob/ob* female mice completely restores gonadotropin secretion, secondary sex organ weight and function, and fertility (Barash et al., 1996; Chehab et al., 1996).

Similar evidence has been obtained in *ob/ob* male mice. Male *ob/ob* mice demonstrate very low levels of fertility (Lane and Dickie, 1954). These animals have low gonadotropin secretion and are hypogonadal (Swerdloff et al., 1978; Mounzih et al., 1997). Seminiferous tubules contain few mature sperm, and

Leydig cells are severely atrophied. As in female *ob/ob* mice, leptin administration to the male mice restores fertility. Seminiferous tubules contain abundant sperm and Leydig cells show normal morphology following leptin treatment (Mounzih et al., 1997).

Thus, reproduction is restored by leptin administration in animals genetically lacking the expression of a functional leptin protein. Undernutrition results in a condition analogous to the *ob/ob* genotype, with inhibited leptin secretion and reproductive function. The effects of undernutrition on reproduction in non-obese animals also can be ameliorated by leptin treatment. The starvation-induced delay in ovulation in non-obese female mice is prevented by leptin treatment (Ahima et al., 1996). Similarly, serum LH and testosterone levels are increased by leptin administration in fasted male mice (Ahima et al., 1996). Restricting nutrition to 80% of ad libitum feed intake causes a >50% reduction in ovarian and uterine weights that is completely prevented by twice-daily injections of leptin (Cheung et al., 1997).

Puberty

As stated above, undernutrition inhibits reproduction in mature animals and delays the onset of puberty as well. Feeding non-obese female rats at 80% of ad libitum intake was found to inhibit the onset of puberty, and no animals showed vaginal openings or estrus at 38 d of age (Cheung et al., 1997). In contrast, normal onset of puberty occurred in rats treated with leptin, even though voluntary feed intake also was 80% that of control animals with ad libitum feed intake (Cheung et al., 1997). Leptin also has been shown to advance puberty in non-obese mice with ad libitum feed intake. Leptin treatment that produced a reduction in food intake and growth by approximately 15% also advanced the onset of puberty by an average of 11 d (47.5 vs 36.5 d in saline vs leptin group; Chehab et al., 1997). In a separate study, leptin treatment, which had no effect on food intake and growth, also advanced the onset of puberty

in mice with ad libitum feed intake, but to a lesser degree (Ahima et al., 1997).

The discovery that leptin treatment can advance reproductive maturation in undernourished and well-fed animals naturally raises the question as to whether leptin plays a role in the normal onset of puberty. The availability of immunoassays for leptin in rodents and humans has permitted evaluations of circulating leptin concentrations in the peripubertal period. Serum leptin concentrations increase during puberty onset in mice (Chehab et al., 1997). Leptin secretion also transiently increases before puberty in boys (Mantzoros et al., 1997). These observations suggest that a causal link between increasing leptin secretion and sexual maturation could exist.

The possibility also exists that increasing leptin sensitivity during early development could exert control over the timing of puberty. When adjusted for body fat, serum leptin concentrations were found to decrease during maturation in children (Hassink et al., 1996). Because elevated serum leptin concentrations often accompany leptin resistance in obesity (Rohner-Jeanrenaud and Jeanrenaud, 1996), the higher levels of leptin secretion in younger children (Hassink et al., 1996) may reflect a relative resistance to leptin. This resistance could serve to maintain high levels of food intake and growth, as well as preventing the premature onset of puberty. Consistent with this premise, a developmental decline in leptin gene expression with no concurrent change in body fat content has been observed in young pigs (Matteri et al., 1997).

Leptin Effects on Reproduction: Mechanism(s) of Action

A logical first step in understanding the mechanisms by which leptin influences reproduction is to determine the location of functional receptors for this hormone. Leptin receptor mRNA has been localized in ventromedial and arcuate hypothalamic nuclei and in anterior pituitary tissue of sheep (Dyer et al., 1997). In rats, leptin receptor mRNA can readily be detected in the ovary, testis, uterus, hypothalamus, and pituitary gland (Schwartz et al., 1996c; Zamorano et al., 1997). Localization of the receptor in reproductive tissues likely occurs across species, because leptin receptor mRNA also is found in human ovaries and testes (Cioffi et al., 1996). Leptin receptor gene expression also has been demonstrated in immortalized rat GnRH neurons and ovarian granulosa cells by RT-PCR analysis (Zamorano et al., 1997).

Thus, leptin could act at multiple sites in the reproductive system. Leptin treatment enhances gonadotropin secretion (Barash et al., 1996) and ovarian side chain cleavage and 17α-hydroxylase mRNA levels (Zamorano et al., 1997). Increased uterine weight in leptin-treated ob/ob mice seems to occur as a result of proliferative responses to increased ovarian estrogen production (Barash et al., 1996). Similarly, trophic responses in seminal vesicles are likely a result of increased testosterone production (Barash et al., 1996). Although the most accepted hypothesis is that the overall leptin-induced stimulation of reproductive function occurs secondarily to increased gonadotropin secretion, the possibility of direct effects cannot be discounted.

The evidence for neuroendocrine effects of leptin on GnRH release is convincing. Increased gonadotropin secretion consistently occurs as a result of leptin treatment in ob/ob mice and undernourished animals. Leptin treatment advances the onset of puberty, which is known to be controlled by hypothalamic release of GnRH. Hypothalamic GnRH release has long been recognized to be deficient in ob/ob mice (Swerdloff et al., 1978; Batt et al., 1982). Leptin stimulates LH release in estrogen-primed ovariectomized rats, indicating stimulatory effects at the level of the anterior pituitary gland and(or) hypothalamus (Yu et al., 1997). Leptin directly stimulates gonadotropin secretion from cultured rat, steer, and pig pituitary cells (Barb et al., 1997; Liou et al., 1997; Yu et al., 1997). Cultured median eminence-arcuate explants from rats release GnRH in response to leptin (Yu et al., 1997). The mechanism whereby leptin directly stimulates gonadotroph function is not known. There is reason to believe that the effects on hypothalamic GnRH release could be mediated by NPY.

Neuropeptide Y is a 36-amino acid neuropeptide found in areas of the hypothalamus involved in food intake and neuroendocrine control. Contrary to leptin, NPY is a potent stimulator of feed intake and inhibitor of gonadotropin secretion (Parrott et al., 1986; Pau et al., 1988; McDonald et al., 1989; Miner et al., 1989; McShane et al., 1992; Kalra and Kalra, 1996). Undernutrition increases NPY gene expression in the arcuate (O'Shea and Gundlach, 1991; McShane et al., 1993; Adam et al., 1997) and elevates cerebrospinal fluid NPY concentrations (Kaye et al., 1990). The increase in NPY production has been postulated to decrease the stimulatory input to downstream neural pathways that ultimately reach the GnRH neurons (Campfield et al., 1996; Schwartz et al., 1996c; Adam et al., 1997; Yu et al. 1997). Leptin administration decreases NPY expression in the arcuate nucleus (Campfield et al., 1996; Schwartz et al., 1996c), presumably removing the inhibition of GnRH release. These data have led to the speculation that receptors for leptin may exist on NPY neurons. In fact, leptin receptors have been localized recently on hypothalamic NPY neurons in mice (Mercer et al., 1996a) and sheep (Duane Keisler, University of Missouri, personal communication). Ob/ob mice that also are homozygous for a recessive mutant NPY allele have been generated (Erickson et al., 1996b). These animals lack leptin and NPY and are less obese and more fertile than ob/ob mice with intact NPY production.

Implications for Reproduction

Even at this early stage of our understanding of leptin biology, there is considerable evidence that leptin regulates reproductive function in neuroendocrine, and perhaps reproductive, tissues. The potential applicability of this knowledge to improve the reproductive efficiency of livestock is open to speculation. However, removal of inhibitory input to the GnRH release mechanism certainly could facilitate reproductive function not only during the pubertal transition, but also during other periods of neuroendocrine quiescence, such as seasonal and postpartum anestrus. Decreasing postpartum leptin production in women could reflect a restoration of sensitivity to leptin conducive to resumption of reproductive capacity (Butte et al., 1997). Obvious applications would also exist in stimulating reproduction during suboptimal nutrition and reduced body condition.

The Cytokinology of Leptin

Protein Structure

Structural characteristics linking the obese gene product, leptin, to the cytokine family are well-established. Results of several studies provide evidence that leptin is related to the family of helical cytokines, which includes IL-2 and growth hormone. Whereas there is no sequence similarity among the members of this family, all contain a distinctive three-dimensional 4-α-helix bundle structure that has been indicated for leptin by threading analysis (Madej et al., 1995), nuclear magnetic resonance techniques (Kline et al., 1997), and the crystal structure of a mutant human leptin (Glu for Trp at position 100; Zhang et al., 1997).

Leptin and Hematopoiesis

Normal cell turnover and the need to increase specific immune cell populations in response to pathogenic and nonpathogenic stimuli make necessary a means of replenishing and increasing blood and immune cell populations. These biological tasks are accomplished by the process called hematopoiesis. Through this process, the entire spectrum of erythroid, myeloid, and lymphoid cell populations are maintained throughout the life span of the animal (Shivdasani and Orkin, 1996; Leitman and Read, 1997). A relatively small pool of pluripotent stem cells capable of self-renewal and differentiation give rise to all hematopoietic cell lineages. Differentiation of the stem cell results in populations of hematopoietic progenitor cells that show progressively greater commitment toward specific lineages and loss of ability for self-renewal. These progenitor cells are capable of marked proliferation as an integrated response to numerous cytokines and growth factors.

The early indications that leptin might function in hematopoiesis arose from the cytokine characteristics of leptin and its receptor, the identification of leptin receptors in hematopoietic tissues, and from the adipocyte-specific expression of the obese gene given that adipocytes are the most abundant stromal cell type in adult human bone marrow (Gimble et al., 1996). Thus, in addition to serving as a localized energy reservoir, the possibility that leptin produced within the marrow might regulate hematopoiesis was raised. Gainsford et al. (1996) documented the coexpression of OB-RL and OB-RS in numerous fetal and adult murine hematopoietic tissues. Furthermore, leptin enhanced cytokine production and parasite phagocytosis by murine peritoneal macrophages, and transfection studies indicated that the OB-RL receptor isoform stimulates proliferation of Ba/F3 cells and differentiation of M1 cells into macrophages. The OB-RS isoform, which lacks the box 1 and box 2 motifs of the hemopoietin receptor family, was nonfunctional in these studies. Ghilardi and Skoda (1997) obtained a similar proliferation response with the Ba/F3 cell line and linked it to activation of JAK2. Leptin has also been shown to stimulate fetal and adult erythroid and myeloid development (Mikhail et al., 1997). Leptin increased macrophage number and granulocyte colony formation, and leptin and erythropoietin acted synergistically to stimulate erythropoiesis. Given these suggested roles of leptin in hematopoiesis, it is interesting to note that db/db mice, which express the truncated form of the leptin receptor, have reduced steady-state levels of some peripheral blood B and T cell populations and that marrow from this genotype has a deficit of lymphopoietic progenitors (Bennett et al., 1996). Such was not the case in ob/ob mice. Thus, the possibility of an alternative ligand was suggested in the case of the ob/ob genotype. There is indeed considerable overlap among cytokines in their biological activities, and one might anticipate that other cytokines would overshadow the absence of leptin or its receptor (OB-RL) in these models. The basis for the apparent discrepancy between the ob/ob and db/db genotypes remains to be determined.

Myriad cytokines and growth factors control the proliferation, survival, differentiation, and activity of immune cells (reviewed by Ellis et al., 1997). Because a number of these regulatory agents are present in milk, interest in their possible roles in the development of neonatal and infant gastrointestinal immunity has been growing. In this regard, it is perhaps quite significant that leptin has been detected in human breast milk (Houseknecht et al., 1997a), and levels in milk correlate with maternal plasma leptin concentrations and maternal adiposity (Houseknecht et al., 1997b). Although temporal relationships between leptin concentrations and the development of neonatal and infant immune function have not been reported, leptin may indeed influence the onset of gut

immunity or functional development. Further, leptin in milk may provide a link between maternal adiposity and neonatal metabolism and growth.

Leptin and the Acute Inflammatory Response

Anorexia and fever are common components of the physiological response to infection and inflammation that are mediated in part by the proinflammatory cytokine milieu within the brain (Johnson, 1997). Thus, because leptin seems to be a powerful negative regulator of food intake and positive regulator of thermogenesis (discussed above), the question as to whether leptin contributes to the anorexia or the increased metabolic rate during infection has been raised. Grunfeld et al. (1996) and Sarraf et al. (1997) determined in rodents that administration of endotoxin and specific proinflammatory cytokines overrides the normal reduction in obese mRNA and blood leptin due to fasting and causes marked parallel increases in mRNA abundance and serum leptin in conjunction with reduced food intake. Ahima et al. (1996) proposed that neuroendocrine adaptations to food deprivation orchestrated by a reduction in circulating leptin may be the primary biological role of this protein. It is therefore necessary to reconcile potential consequences of a lack of such adaptations in immunologically challenged animals in which leptin concentrations are increased despite food deprivation. However, it should also be noted that a response similar to that described for rodents may not occur in pigs. Leptin mRNA abundance is not influenced, at least acutely (4 h), by endotoxin challenge despite clear clinical indications of a strong acute inflammatory response to the endotoxin in young pigs (M. E. Spurlock, personal communication).

All things considered, it may well be that an increase in the circulating leptin concentration in response to infection or inflammation is multipurposed, encompassing regulation of food intake, metabolic rate, macrophage function, and induction of immune cell proliferation or differentiation. It will be interesting to determine whether the immunological responses to infection influenced by leptin are different in *db/db* or *ob/ob* mice.

Potential Medical Applications for Leptin

Pharmaceutical companies have invested heavily in leptin since 1995. In June 1997, Amgen, a licensee of the Freidman and Rockefeller University leptin patents, announced the successful completion of their Phase 1 regulatory trials with a leptin injectable product. They will be moving on to "... Phase 2 testing of leptin both for weight reduction in obese patients and for those obese patients with the form of diabetes known as non-insulin dependent diabetes mellitus or NIDDM" (Business Wire, June 17, 1997). They reported that there is a dose range in which leptin seemed to be safe for use in humans, but there were injection site reactions, especially with multiple daily injections at the higher doses tested. It is likely that other product concepts will soon be tested in clinical trials to take advantage of the highly desirable effects of leptin on obesity. More complex leptin formulations or other molecules that act on some component of the leptin axis are likely to be in the pipeline soon, and these would be expected to be easier for patients to use than daily injections. The commercial opportunities are some of the largest in the health care arena and thus have attracted huge investments that have driven the tremendous growth in the understanding of this exciting new component of energy balance regulation and obesity.

Applications for Animal Agriculture

Although obesity per se is not a major problem for animal agriculture, improvement of productive efficiency, carcass composition, and animal health and well-being are important goals. The published literature concerning leptin biology in livestock species is small; however, the porcine leptin gene has been cloned (Bidwell et al., 1997) and multiple abstracts and papers are in press. If leptin biology is similar for livestock species and human and rodent species, it is clear that leptin has myriad effects on tissues and endocrine systems that ultimately lead to the coordination of whole-body energy metabolism. Thus, leptin is a homeorhetic hormone that may have a major impact on the performance and well-being of livestock species. Leptin may be classified as a "metabolism modifier"; thus, we can predict that the manipulation of leptin expression and(or) action will be of interest to scientists and pharmaceutical companies wishing to improve productive performance of animals. Furthermore, if leptin is involved in animal responses to disease or stress, the manipulation of leptin action may become an important therapy in the treatment of animal disease.

Direct leptin treatment will not be feasible in livestock production unless affordable, potent analogs or delivery systems are developed. Until then, successful enhancement of reproductive function or manipulation of nutrient partitioning are more likely to be achieved through regulation of leptin production or sensitivity to leptin through nutritional or metabolic manipulation. Genetic selection also could be used to this end. Expressed (Matteri et al., 1998) and intronic (Sasaki et al., 1996) polymorphisms in the leptin gene that may be useful in RFLP-based selection have been discovered.

Implications

Meeting the challenge of optimizing productive efficiency, product quality, and animal health and well-being requires a thorough understanding of the

mechanisms that regulate and coordinate feed intake and energy metabolism in food animals. Data, largely from rodent and human studies, indicate that leptin may play a vital role in coordinating feed intake, energy expenditure, and tissue nutrient utilization under many physiological and pathological conditions. Extensive studies are needed to determine the importance of leptin in the physiology and productivity of food animals.

Literature Cited

Adam, C. L., P. A. Finddlay, C. E. Kyle, P. Young, and J. G. Mercer. 1997. Effect of chronic food restriction on pulsatile luteinizing hormone secretion and hypothalamic neuropeptide Y gene expression in castrate male sheep. J. Endocrinol. 152:329–337.

Ahima, R. S., J. Dshay, S. N. Flier, D. Prabakaran, and J. S. Flier. 1997. Leptin accelerates the onset of puberty in normal female mice. J. Clin. Invest. 99:391–395.

Ahima, R. S., D. Prabakaran, C. Mantzoros, D. Qu, B. Lowell, E. Maratos-Flier, and J. S. Flier. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252.

Armstrong, J. D., and J. H. Britt. 1987. Nutritionally-induced anestrus in gilts: Metabolic and endocrine changes associated with cessation and resumption of estrous cycles. J. Anim. Sci. 65:508–523.

Asdell, S. A. 1949. Nutrition and the treatment of sterility in dairy cattle: A review. J. Dairy Sci. 32:60–70.

Aubert, M. L., and P. C. Sizonenko. 1996. Environmental factors and sexual maturation in rodents. Acta Pediatr. 417:86–88.

Azain, M. J., T. Wang, M. G. Hulsey, D. L. Hartzell, and C. A. Baile. 1997. Adipose tissue-specific effects of intracerebroventricular leptin in rats. J. Anim. Sci. 75:(Suppl. 1)167 (Abstr.).

Banks, W. A., A. J. Kastin, W. Huang, J. B. Jaspan, and L. M. Maness. 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17:305–311.

Barash, I. A., C. C. Cheung, D. S. Weigle, H. Ren, E. B. Kabigting, J. L. Kuijper, D. K. Clifton, and R. A. Steiner. 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144–3147.

Barb, C. R., J. B. Barret, R. R. Kraeling, G. B. Rampacek, X. Yan, and T. G. Ramsay. 1997. Leptin modulation of luteinizing hormone (LH) secretion by pig pituitary cells in culture. 5th Int. Conf. Pig Reprod. p 81 (Abstr.).

Batt, R., D. Everard, G. Gillies, M. Wilkinson, C. Wilson, and T. Yeo. 1982. Investigation into the hypogonadism of the obese mouse (genotype ob/ob). J. Reprod. Fertil. 64:363–371.

Beltranena, E., F. X. Aherne, and G. R. Foxcroft. 1993. Innate variability in sexual development irrespective of body fatness in gilts. J. Anim. Sci. 71:471–480.

Bennett, B. D., G. P. Solar, J. Q. Yuan, J. Mathias, G. R. Thomas, and W. Matthews. 1996. A role for leptin and its cognate receptor in hematopoiesis. Curr. Biol. 6:1170–1180.

Berti, L., M. Kellerer, E. Capp, and H. U. Haring. 1997. Leptin stimulates glucose transport and glycogen synthesis in C_2C_{12} myotubes: Evidence for a PI3-kinase mediated effect. Diabetologia 40:606–609.

Bidwell, C. A., S. Ji, G. R. Frank, S. G. Cornelius, G. M. Willis, and M. E. Spurlock. 1997. Cloning and expression of the porcine *obese* gene. Anim. Biotechnol. 8:191–206.

Billington, C. J., J. E. Briggs, M. Grace, and A. S. Levine. 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am. J. Physiol. 260:R321–R327.

Bonner, J. C., and A. R. Brody. 1995. Cytokine-binding proteins in the lung. Am. J. Physiol. 268:L869–L878.

Bornstein, S. R., K. Uhlmann, A. Haidan, M. Ehrhart-Bornstein, and W. A. Scherbaum. 1997. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland. Leptin inhibits cortisol release directly. Diabetes 46:1235–1238.

Bronson, F. H. 1986. Food-restricted, prepubertal, female rats: Rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone. Endocrinology 118:2483–2487.

Brown, B. W. 1994. A review of nutritional influences on reproduction in boars, bulls and rams. Reprod. Nutr. Dev. 34:89–114.

Butte, N. F., J. M. Hopkinson, and M. A. Nicolson. 1997. Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. J. Clin. Endocrinol. Metab. 82:585–589.

Cameron, J. L., D. L. Helmreich, and D. A. Schreihofer. 1993. Modulation of reproductive hormone secretion by nutritional intake: Stress signals versus metabolic signals. Human Reprod. 8:162–167.

Campfield, L. A., F. J. Smith, and P. Burn. 1996. The OB protein (leptin) pathway-A link between adipose tissue mass and central neural networks. Horm. Metab. Res. 28:619–632.

Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos, and P. Burn. 1995. Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science (Wash DC) 269:546–549.

Caro, J. F., J. W. Kolaczynski, M. R. Nyce, J. P. Ohannesian, I. Opentanova, W. H. Goldman, R. B. Lynn, P. L. Zhang, M. K. Sinha, and R. V. Considine. 1996a. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: A possible mechanism for leptin resistance. Lancet 348:159–161.

Caro, J. F., M. K. Sinha, J. W. Kolaczynski, P. L. Zhang, and R. V. Considine. 1996b. Leptin: The tale of an obesity gene. Diabetes 45:1455–1462.

Carro, E., R. Senaris, R. V. Considine, F. F. Casanueva, and C. Dieguez. 1997. Regulation of in vivo growth hormone secretion by leptin. Endocrinology 138:2203–2206.

Chehab, F. F., M. E. Lim, and R. Lu. 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat. Genet. 12:318–320.

Chehab, F. F., K. Mounzih, R. Lu, and M. E. Lim. 1997. Early onset of reproductive function in normal female mice treated with leptin. Science (Wash DC) 275:88–90.

Chen, G., K. Koyama, X. Yuan, Y. Lee, Y. Zhou, R. O'Doherty, C. B. Newgard, and R. H. Unger. 1996a. Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proc. Natl. Acad. Sci. USA 93:14795–14799.

Chen, H., O. Charlat, L. A. Tartaglia, E. A. Woof, X. Weng, S. J. Ellis, N. D. Lakey, J. Culpepper, K. J. Moore, R. E. Breitbart, G. M. Duyk, R. I. Tepper, and J. P. Morgenstern. 1996b. Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in db/db mice. Cell 84:491–495.

Cheung, C. C., J. E. Thornton, J. L. Kuijper, D. S. Weigle, D. K. Clifton, and R. A. Steiner. 1997. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology 138:855–858.

Christy, R. J., V. W. Yang, J. M. Ntambi, D. E. Geiman, W. M. Landschulz, A. D. Friedman, Y. Nakabeppu, T. J. Kelly, and M. D. Lane. 1989. Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. Genes Dev. 3:1323–1335.

Chua, S. C., W. K. Chung, X. S. Wu-Peng, Y. Zhang, S. M. Liu, L. Tartaglia, and R. L. Leibel. 1996. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (Leptin) receptor. Science (Wash DC) 271:994–996.

Cioffi, J. A., A. W. Shafer, T. J. Zupancic, J. Smith-Gbur, A. Mikhail, D. Platika, and H. R. Snodgrass. 1996. Novel B219/OB receptor isoforms:Possible role of leptin in hematopoiesis and reproduction. Nat. Med. 2:585–593.

Cohen, B., D. Novick, and M. Rubinstein. 1996. Modulation of insulin activities by leptin. Science (Wash DC) 274:1185–1188.

Colditz, G. A. 1992. Economic costs of obesity. Am. J. Clin. Nutr. 55:503S–507S.

Coleman, D. L. 1973. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9:294–298.

Coleman, D. L., and K. P. Hummel. 1969. Effects of parabiosis of normal with genetically diabetic mice. Am. J. Physiol. 217:1298–1304.

Collins, S., C. M. Kuhn, A. E. Petro, A. G. Swick, B. A. Chrunyk, and R. W. Surwit. 1996. Cross-talk between white and brown fat: Leptin increases sympathetic outflow to brown adipose tissue. 10th Int. Congr. Endocrinol. 1:348 (Abstr.).

Considine, R. V., M. Sinha, M. Heiman, A. Kriauciunas, T. Stephens, M. Nyce, J. Ohannesian, and C. Marco. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334:292–295.

Cusin, I., F. Rohner-Jeanrenaud, A. Stricker-Krongrad, and B. Jeanrenaud. 1996. The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats. Reduced sensitivity compared with lean animals. Diabetes 45:1446–1450.

Cusin, I., A. Sainsbury, P. Doyle, F. Rohner-Jeanrenaud, and B. Jeanrenaud. 1995. The ob gene and insulin. A relationship leading to clues to the understanding of obesity. Diabetes 44:1467–1470.

De Vos, P., R. Saladin, J. Auwerx, and B. Staels. 1995. Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. J. Biol. Chem. 270:15958–15961.

Devos, R., J. G. Richards, L. A. Campfield, L. A. Tartaglia, Y. Guisez, J. Van der Heyden, J. Travernier, G. Plaetinck, and P. Burn. 1996. OB protein binds specificially to the choroid plexus of mice and rats. Proc. Natl. Acad. Sci. USA 93:5668–5673.

Diamond, F. B., D. C. Eichler, G. Duckett, E. V. Jorgensen, D. Shulman, and A. W. Root. 1997. Demonstration of a leptin binding factor in human serum. Biochem. Biophys. Res. Commun. 233:818–822.

Dryden, S., and G. Williams. 1996. The role of hypothalamic peptides in the control of energy balance and body weight. Curr. Opin. Endocrinol. Diabetes 3:51–58.

Dyer, C. J., J. M. Simmons, R. L. Matteri, and D. H. Keisler. 1997. Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. Domest. Anim. Endocrinol. 14:119–128.

Ellis, L. A., A. M. Mastro, and M. F. Picciano. 1997. Do milk-borne cytokines and hormones influence neonatal immune cell function? J. Nutr. 127:985S–988S.

Elmquist, J. K., R. S. Ahima, E. Maratos-Flier, J. S. Flier, and C. B. Saper. 1997. Leptin activates neurons in ventrobasal hypothalamus and brainstem. Endocrinology 138:839–842.

Emilsson, V., Y. Liu, M. A. Cawthorne, N. M. Morton, and M. Davenport. 1997. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes 46:313–316.

Erickson, J. C., K. E. Clegg, and R. D. Palmiter. 1996a. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature (Lond.) 381:415–418.

Erickson, J. C., G. Hollopeter, and R. D. Palmiter. 1996b. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science (Wash DC) 274:1704–1707.

Foster, D. L., and D. H. Olster. 1985. Effect of restricted nutrition on puberty in the lamb: Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. Endocrinology 116:375–381.

Frederich, R. C., A. Hamann, S. Anderson, B. Lollmann, B. B. Lowell, and J. S. Flier. 1995a. Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. Nat. Med. 1:1311–1314.

Frederich, R. C., B. Lollmann, A. Hamann, A. Napolitano-Rosen, B. B. Kahn, B. B. Lowell, and J. S. Flier. 1995b. Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J. Clin. Invest. 96:1658–1663.

Freytag, S. O., and T. J. Geddes. 1992. Reciprocal regulation of adipogenesis by Myc and C/EBP alpha. Science (Wash DC) 256:379–382.

Gainsford, T., T. A. Willson, D. Metcalf, E. Handman, C. McFarlane, A. Ng, N. A. Nicola, W. S. Alexander, and D. J. Hilton. 1996. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc. Natl. Acad. Sci. USA 93:14564–14568.

Gettys, T. W., P. J. Harkness, and P. M. Watson. 1996. The β_3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. Endocrinology 137:4054–4057.

Ghilardi, N., and R. C. Skoda. 1997. The leptin receptor activates Janus Kinase 2 and signals for proliferation in a factor-dependent cell line. Mol. Endocrinol. 11:393–399.

Ghilardi, N., S. Ziegler, A. Wiestner, R. Stoffel, M. H. Heim, and R. C. Skoda. 1996. Defective STAT signaling by the leptin receptor in diabetic mice. Proc. Natl. Acad. Sci. USA 93:6231–6235.

Gimble, J. M., C. E. Robinson, X. Wu, and K. A. Kelly. 1996. The function of adipocytes in the bone marrow stroma: An update. Bone 19:421–428.

Glaum, S. R., M. Hara, V. P. Bindokas, C. C. Lee, K. S. Polonsky, G. I. Bell, and R. J. Miller. 1996. Leptin, the obese gene product, rapidly modulates synaptic transmission in the hypothalamus. Mol. Pharmacol. 50:230–235.

Gong, D. W., S. Bi, R. E. Pratley, and B. D. Weintraub. 1996. Genomic structure and promoter analysis of the human obese gene. J. Biol. Chem. 271:3971–3974.

Grunfeld, C., C. Zhao, J. Fuller, A. Pollock, A. Moser, J. Friedman, and K. R. Feingold. 1996. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. A role for leptin in the anorexia of infection. J. Clin. Invest. 97:2152–2157.

Gura, T. 1997. Obesity sheds its secrets. Science (Wash DC) 275:751–753.

Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lalone, S. K. Burley, and J. M. Friedman. 1995. Weight reducing effects of the plasma protein encoded by the obese gene. Science (Wash DC) 269:543–546.

Hamann, A., and S. Matthaei. 1996. Regulation of energy balance by leptin. Exp. Clin. Endocrinol. Diabetes 104:293–300.

Hardie, L. J., N. Guilhot, and P. Trayhurn. 1996. Regulation of leptin production in cultured mature white adipocytes. Horm. Metab. Res. 28:685–689.

Hassink, S. G., D. V. Sheslow, E. de Lancy, I. Opentanova, R. V. Considine, and J. F. Caro. 1996. Serum leptin in children with obesity: Relationship to gender and development. Pediatrics 98:201–203.

Hausberger, F. X. 1959. Parabiosis and transplantation experiments in hereditarily obese mice. Anat. Rec. 130:313.

He, Y., H. Chen, M. J. Quon, and M. Reitman. 1995. The mouse obese gene. Genomic organization, promoter activity, and activation by CCAAT/enhancer-binding protein alpha. J. Biol. Chem. 270:28887–28891.

Heaney, M. L., and D. W. Golde. 1993. Soluble hormone receptors. Blood 82:1945–1948.

Herrera, R., H. S. Ro, K. G. Robinson, K. G. Xanthopoulos, and B. M. Spiegelman. 1989. A direct role for C/EBP and the AP-1 binding site in gene expression linked to adipocyte differentiation. Mol. Cell. Biol. 9:5331–5339.

Hervey, G. R. 1958. The effects of lesions in the hypothalamus in parabiotic rats. J. Physiol. 145:336–352.

Hollenberg, A. N., V. S. Susulic, J. P. Madura, B. Zhang, D. E. Moller, P. Tontonoz, P. Sarraf, B. M. Spiegelman, and B. B. Lowell. 1997. Functional antagonism between CCAAT/enhancer binding protein-α and peroxisome proliferator-activated receptor-γ on the leptin promoter. J. Biol. Chem. 272:5283–5290.

Houseknecht, K. L., S. N. Flier, R. C. Frederich, E. U. Frevert, J. S. Flier, and B. B. Kahn. 1996a. Secretion of leptin and TNF-α by the adipocyte in vitro: Regulation with genetic and dietary-induced obesity. J. Anim. Sci. 74(Suppl. 1):81 (Abstr.).

Houseknecht K. L., S. N. Flier, E. U. Frevert, R. C. Frederich, J. S. Flier, and B. B. Kahn. 1996b. Leptin secretion correlates with adipocyte size in genetic and dietary obesity. Diabetes 45: (Suppl. 2)41A (Abstr.).

Houseknecht, K. L., C. S. Mantzoros, R. Kuliawat, E. Hadro, J. S. Flier, and B. B. Kahn. 1996c. Evidence for leptin binding to proteins in serum of rodents and humans: Modulation with obesity. Diabetes 45:1638–1643.

Houseknecht, K. L., M. K. McGuire, M. A. McGuire, C. P. Portocarrero, T. D. Shultz, and Y. S. Park. 1997a. Evidence that leptin is present in human breast milk. FASEB J. 11:A238 (Abstr.).

Houseknecht, K. L., M. K. McGuire, C. P. Portocarrero, M. A. McGuire, and K. Beerman. 1997b. Leptin is present in human milk and is related to maternal plasma leptin concentration and adiposity. Biochem. Biophys. Res. Commun. 240:742–747.

Howland, B. E. 1971. Gonadotropin levels in female rats subjected to restriced feed intake. J. Reprod. Fertil. 27:467–470.

Hummel, K. P., M. M. Dickie, and D. L. Coleman. 1966. Diabetes, a new mutation in the mouse. Science (Wash DC) 153: 1127–1128.

Hwang, C., S. Mandrup, O. A. MacDougald, D. E. Geiman, and M. D. Lane. 1996. Transcriptional activation of the mouse obese (Ob) gene by CCAAT/enhancer binding protein α. Proc. Natl. Acad. Sci. USA 93:873–877.

Ingalls, A. M., M. M. Dickie, and G. D. Snell. 1950. Obese, a new mutation in the mouse. J. Hered. 41:317–318.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci. 75:1244–1255.

Kallen, C. B., and M. A. Lazar. 1996. Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. 93:5793–5796.

Kalra, S. P., and P. S. Kalra. 1996. Nutritional infertility–The role of the interconnected hypothalamic neuropeptide Y-Galanin-Opiod network. Front. Neuroendocrinol. 17:371–401.

Kaye, W. H., W. Berrettini, H. E. Gwirtsman, and D. T. George. 1990. Altered cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. Arch. Gen. Psychiatry 47:548–556.

Keane, M. G. 1975. Effect of age and plane of nutrition during breeding on reproductive performance of Suffolk-X ewe lambs. Ir. J. Agric. Res. 14:91–98.

Kennedy, G. C. 1953. The role of depot fat in the hypothalamic control of food intake in the rat. Proc. R. Soc. 140:578–592.

Kennedy, G. C., and J. Mitra. 1963. Body weight and food intake as initiating factors for puberty in the rat. J. Physiol. 166:408–416.

Kieffer, T. J., R. S. Heller, and J. F. Habener. 1996. Leptin receptors expressed on pancreatic b-cells. Biochem. Biophys. Res. Commun. 224:522–527.

Kieffer, T. J., R. S. Heller, C. A. Leech, G. G. Holz, and J. F. Habener. 1997. Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic b-cells. Diabetes 46:1087–1093.

Kirkwood, R. N., and F. X. Aherne. 1985. Energy intake, body composition and reproductive performance of the gilt. J. Anim. Sci. 60:1518–1529.

Kline, A. D., G. W. Becker, L. M. Churgay, B. E. Landen, D. K. Martin, W. L. Muth, R. Rathnachalam, J. M. Richardson, B. Schoner, M. Ulmer, and J. E. Hale. 1997. Leptin is a four-helix bundle: Secondary structure by NMR. FEBS Lett. 407:239–242.

Kolaczynski, J. W., R. V. Considine, J. P. Ohannesian, C. Marco, I. Opentanova, M. R. Nyce, M. Myint, and J. F. Caro. 1996a. Responses of leptin to short-term fasting and refeeding in humans. A link with ketogenesis but not ketones themselves. Diabetes 45:1511–1515.

Kolaczynski, J. W., J. P. Ohannesian, R. V. Considine, C. C. Marco, and J. F. Caro. 1996b. Response of leptin to short-term and prolonged overfeeding in humans. J. Clin. Endocrinol. Metab. 81:4162–4165.

Kroder, G., M. Kellerer, and H. U. Haring. 1996. Effect of leptin on insulin signalling in rat-1 fibroblasts overexpressing HIR. Exp. Clin. Endocrinol. Diabetes 104(Suppl. 2):66 (Abstr.).

Landefeld, T. D., F.J.P. Ebling, J. M. Suttie, L. A. Vannerson, V. Padmanabhan, I. A. Beitins, and D. L. Foster. 1989. Metabolic interfaces between growth and reproduction. II. Characterization of changes in messenger ribonucleic acid concentrations of gonadotropin subunits, growth hormone, and prolactin in nutritionally growth-limited lambs and the differential effects of increased nutrition. Endocrinology 125:351–356.

Lane, P. W., and M. M. Dickie. 1954. Fertile obese male mice. Relative sterility in obese males corrected by dietary restriction. J. Hered. 45:56–58.

Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee, and J. M. Friedman. 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature (Lond.) 379: 632–635.

Lehmann, J. M., L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J. Biol. Chem. 270: 12953–12956.

Leitman, S. F., and E. J. Read. 1997. Hematopoietic progenitor cells. Semin. Hematol. 83:341–358.

Levin, N., C. Nelson, A. Gurney, R. Vadlen, and F. J. de Sauvage. 1996. Decreased food intake does not completely account for adiposity reduction after ob protein infusion. Proc. Natl. Acad. Sci. USA 93:1726–1730.

Licinio, J., C. Mantzoros, A. B. Negrao, G. Cizza, M. Wong, P. B. Bongiorno, G. P. Chrousos, B. Karp, C. Allen, J. S. Flier, and P. W. Gould. 1997. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat. Med. 3: 575–579.

Liou, S. S., J. M. Lim, R. M. Blair, and W. Hansel. 1997. Leptin causes release of LH and FSH from perfused murine and bovine pituitary glands. Biol. Reprod. 56:(Suppl. 1)171 (Abstr.).

MacDougald, O. A., C. Hwang, H. Fan, and M. D. Lane. 1995. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. 92:9034–9037.

Madej, T., M. S. Boguski, and S. H. Bryant. 1995. Threading analysis suggests that the obese gene product may be a helical cytokine. FEBS Lett. 373:13–18.

Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P. A. Kern, and J. M. Friedman. 1995. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1:1155–1161.

Mantzoros, C. S., D. Qu, R. C. Frederich, V. S. Susulic, B. B. Lowell, E. Maratos-Flier, and J. S. Flier. 1996. Activation of beta3 adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. Diabetes 45:909–914.

Mantzoros, C. S., J. S. Flier, and A. D. Rogol. 1997. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J. Clin. Endocrinol. Metab. 82:1066–1070.

Marks, J. L., M. Li, M. Schwartz, D. Porte, and D. G. Baskin. 1992. Effect of fasting on regional levels of neuropeptide Y mRNA and insulin receptors in the rat hypothalamus: an autoradiographic study. Mol. Cell. Neurosci. 3:199–205.

Matteri, R. L., J. A. Carroll, J. E. Monnig, J. A. Sterle, T. L. Veum, and W. R. Lamberson. 1998. Polymorphisms of porcine leptin cDNA. J. Anim. Sci. 76(Suppl. 1) (Abstr.).(In press).

Matteri, R. L., J. A. Carroll, T. L. Veum, R. S. MacDonald, J. A. Pardalos, and L. S. Hillman. 1997. Leptin and growth hormone (GH) receptor gene expression in adipose tissue of young pigs treated with growth hormone. J. Anim. Sci. 75:(Suppl.1)215 (Abstr.).

McDonald, J. K., M. L. Lumpkin, and L. V. DePaulo. 1989. Neuropeptide-Y suppresses pulsatile secretion of leuteinizing hormone in ovariectormized rats: Possible site of action. Endocrinology 125:186–191.

McShane, T. M., T. May, J. L. Miner, and D. H. Keisler. 1992. Central actions of neuropeptide Y may provide a neuromodulatory link between nutrition and reproduction. Biol. Reprod. 46: 1151–1157.

McShane, T. M., S. L. Peterson, S. McCrone, and D. H. Keisler. 1993. Influence of food restriction on neuropeptide-Y, proopiomelanocortin, and luteinizing hormone-releasing hormone gene expression in sheep hypothalami. Biol. Reprod. 49: 831–839.

Mercer, J. G., N. Hoggard, L. M. Williams, C. B. Lawrence, L. T. Hannah, P. J. Morgan, and P. Trayhurn. 1996a. Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. J. Neuroendocrinol. 8: 733–735.

Mercer, J. G., N. Hoggard, L. M. Williams, C. B. Lawrence, L. T. Hannah, and P. Trayhurn. 1996b. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Lett. 387:113–116.

Mikhail, A., E. X. Beck, A. Shafer, B. Barut, J. Smith Gbur, T. J. Zupancic, A. C. Schweitzer, J. A. Cioffi, G. Lacuaud, B. Ooyang, G. Keller, and H. R. Snodgrass. 1997. Leptin stimulates fetal and adult erythroid and myeloid development. Blood 89: 1507–1512.

Miner, J. L., M. A. Della-Ferra, J. A. Paterson, and C. A. Baile. 1989. Lateral cerebroventricular injection of neuropeptide Y stimulated feeding in sheep. Am. J. Physiol. 257:R383–R387.

Montague, C. T., I. S. Farooqi, J. P. Whitehead, M. A. Soos, H. Rau, N. J. Wareham, C. P. Sewter, J. E. Digby, S. N. Mohammed, J. A. Hurst, C. H. Cheetham, A. R. Earley, A. H. Barnett, J. B. Prins, and S. O'Rahilly. 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature (Lond.) 387:903–908.

Mounzih, K., R. Lu, and F. F. Chehab. 1997. Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology 138:1190–1193.

Muller, G., J. Ertl, M. Gerl, and G. Preibisch. 1997. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J. Biol. Chem. 272:10585–10593.

Muoio, D. M., G. L. Dohn, F. T. Fiedorek, E. B. Tapscott, and R. A. Coleman. 1997. Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 46:1360–1363.

Nakashima, K., M. Narazaki, and T. Taga. 1997. Leptin receptor (OB-R) oligomerizes with itself but not with its closely related cytokine signal transducer gp130. FEBS Lett. 403:79–82.

Nolan, J. J., J. M. Olefsky, M. R. Nyce, R. V. Considine, and J. F. Caro. 1996. Effect of troglitazone on leptin production. Studies in vitro and in human subjects. Diabetes 45:1276–1278.

O'Shea, R. D., and A. L. Gundlach. 1991. Preproneuropeptide Y messenger ribonucleic acid in the hypothalamic arcuate nucleus is increased by food deprivation or dehydration. J. Neuroendocrinol. 3:11–14.

Parrott, R. F., R. P. Heavens, and B. A. Baldwin. 1986. Stimulation of feeding in the satiated pig by intracerebroventricular injection of neuropeptide Y. Physiol. Behav. 36:523–525.

Pau, M.Y.C., K.Y.F. Pau, and H. G. Spies. 1988. Characterization of central actions of neuropeptide Y on food and water intake in rabbits. Physiol. Behav. 44:797–802.

Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science (Wash DC) 269:540–543.

Pettigrew, J. E., and M. D. Tokach. 1991. Nutrition and female reproduction. Pig News Info. 12:559–562.

Rentsch, J., N. Levens, and M. Chiesi. 1995. Recombinant ob-gene product reduces food intake in fasted mice. Biochem. Biophys. Res. Commun. 214:131–136.

Rohner-Jeanrenaud, F., and B. Jeanrenaud. 1996. Obesity, leptin and the brain. New Engl. J. Med. 334:234–235.

Rosenblum, C. I., M. Tota, D. Cully, T. Smith, R. Collum, S. Qureshi, J. F. Hess, M. S. Phillips, P. J. Hey, A. Vongs, T. M. Fong, L. Xu, H. Y. Chen, R. G. Smith, C. Schindler, and L.H.T. Van der Ploeg. 1996. Functional STAT1 and 3 signaling by the leptin receptor (OB-R); reduced expression of the rat fatty receptor in transfected cells. Endocrinology 137:5178–5181.

Saladin, R., P. De Vos, M. Guerre-Millo, A. Leturque, J. Girard, B. Staels, and J. Auwerx. 1995. Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527–529.

Saltiel, A., and J. M. Olefsky. 1996. Thiazolidinediones in the treatment of insulin resistance and Type II diabetes. Diabetes 45: 1661–1669.

Sarraf, P., R. C. Frederich, E. M. Turner, G. Ma, N. T. Jaskowiak, D. J. Rivet, J. S. Flier, B. B. Lowell, D. L. Fraker, and H. R. Alexander. 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: Potential role in inflammatory anorexia. J. Exp. Med. 185:171–175.

Sasaki, S., A. C. Clutter, and D. Pomp. 1996. Assignment of the porcine obese (leptin) gene to chromosome 18 by linkage analysis of a new PCR-based polymorphism. Mamm. Genome 7: 471–472.

Schoonjans, K., B. Staels, and J. Auwerx. 1996a. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J. Lipid Res. 37:907–925.

Schoonjans, K., B. Staels, and J. Auwerx. 1996b. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim. Biophys. Acta 1302:93–109.

Schwartz, M. W., D. G. Baskin, T. R. Bukowski, J. L. Kujiper, D. Foster, G. Lasser, D. E. Prunkard, D. Porte, S. C. Woods, R. J. Seeley, and D. S. Weigle. 1996a. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 45:531–535.

Schwartz, M. W., E. Peskind, M. Raskind, E. J. Boyko, and D. Porte. 1996b. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nat. Med. 2:589–593.

Schwartz, M. W., R. J. Seeley, L. A. Campfield, P. Burn, and D. G. Baskin. 1996c. Identification of targets of leptin action in rat hypothalamus. J. Clin. Invest. 98:1101–1106.

Shivdasani, R. A., and S. H. Orkin. 1996. The transcriptional control of hematopoiesis. J. Am. Soc. Hematol. 87:4025–4039.

Short, R. E., and R. A. Bellows. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J. Anim. Sci. 32:127–131.

Sinha, M. K., J. P. Ohannesian, M. L. Heiman, A. Kriauciunas, T. W. Stephens, S. Magosin, C. Marco, and J. F. Caro. 1996a. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J. Clin. Invest. 97:1344–1347.

Sinha, M. K., I. Opentanova, J. P. Ohannesian, J. W. Kolaczynski, M. L. Heiman, J. Hale, G. W. Becker, R. R. Bowsher, T. W. Stephens, and J. F. Caro. 1996b. Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects during short-term fasting. J. Clin. Invest. 98:1277–1282.

Slieker, L. J., K. W. Sloop, P. L. Surface, A. Kriauciunas, F. LaQuier, J. Manetta, J. Bue-Valleskey, and T. W. Stephens. 1996. Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J. Biol. Chem. 271:5301–5304.

Spiegelman, B. M., and J. S. Flier. 1996. Adipogenesis and obesity: Rounding out the big picture. Cell 87:377–389.

Stephens, T. W., M. Basinski, P. K. Bristow, J. M. Bue-Valleskey, S. G. Burgett, L. Craft, J. Hale, J. Hoffmann, H. M. Hsiung, A. Kriauciunas, W. MacKellar, P. R. Rosteck, Jr., B. Schoner, D. Smith, F. C. Tinsley, X. Y. Zhang, and M. Heiman. 1995. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature (Lond.) 377:530–532.

Swerdloff, R. S., M. Peterson, A. Vera, R. A. Batt, D. Hever, and G. A. Bray. 1978. The hypothalamic-pituitary axis in genetically obese (ob/ob) mice: Response to luteinizing hormone-releasing hormone. Endocrinology 103:542–547.

Takahashi, Y., Y. Okimura, I. Mizuno, H. Takahashi, H. Kaji, T. Uchiyama, H. Abe, and K. Chihara. 1996. Leptin induces tyrosine phosphorylation of cellular proteins including STAT-1 in human renal adenocarcinoma cells, ACHN. Biochem. Biophys. Res. Commun. 228:859–864.

Tartaglia, L. A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark, J. Deeds, C. Muir, S. Sanker, A. Moriarty, K. J. Moore, J. S. Smutko, G. G. Mays, E. A. Woolf, C. A. Monroe, and R. I. Tepper. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263–1271.

Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPARγ2: Tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8:1224–1234.

Trayhurn, P., J. S. Duncan, D. V. Rayner, and L. J. Hardie. 1996. Rapid inhibition of ob gene expression and circulating leptin levels in lean mice by the b3-adrenoceptor agonists BRL 35135A and ZD2079. Biochem. Biophys. Res. Commun. 228:605–610.

Trayhurn, P., M.E.A. Thomas, J. S. Duncan, and D. V. Rayner. 1995. Effects of fasting and refeeding on Ob gene expression in white adipose tissue of lean and obese mice. FEBS Lett. 368:488–490.

Umek, R. M., A. D. Friedman, and S. L. McKnight. 1991. CCAAT/enhancer binding protein: A component of a differentiation switch. Science (Wash DC) 251:288–292.

Utriainen, T., R. Malmstrom, S. Makimattila, and H. Yki-Jarvinen. 1996. Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. Diabetes 45:1364–1366.

Vaisse, C., J. L. Halaas, C. M. Horvath, J. E. Darnell, M. Stoffel, and J. M. Friedman. 1996. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat. Gen. 14:95–97.

Vidal, H., D. Auboeuf, P. De Vos, B. Staels, J. P. Riou, J. Auwerx, and M. Laville. 1996. The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. J. Clin. Invest. 98:251–255.

Vigersky, R. A., A. E. Andersen, R. H. Thompson, and D. L. Loriaux. 1977. Hypothalamic dysfunction in secondary amenorrhea associated with simple weight loss. N. Engl. J. Med. 297:1141–1145.

Wabitsch, M., P. B. Jensen, W. F. Blum, C. T. Christoffersen, P. Englaro, E. Heinze, W. Rascher, W. Teller, H. Tornqvist, and H. Hauner. 1996. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45:1435–1438.

Watowich, S. S., H. Wu, M. Socolovsky, U. Klingmuller, S. N. Constantinescu, and H. F. Lodish. 1996. Cytokine receptor signal transduction and the control of hemoatopoietic cell development. Annu. Rev. Cell Dev. Biol. 12:91–128.

Weigle, D. S., T. R. Bukowski, D. C. Foster, S. Holderman, J. M. Kramer, G. Lasser, C. E. Lofton-Day, D. E. Prunkard, C. Raymond, and J. L. Kujiper. 1995. Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. J. Clin. Invest. 96:2065–2070.

White, D. W., K. K. Kuropatwinski, R. Devos, H. Baumann, and L. A. Tartaglia. 1997. Leptin receptor (OB-R) signaling. cytoplasmic domain mutational analysis and evidence for receptor homo-oligmerization. J. Biol. Chem. 272:4065–4071.

White, D. W., and L. A. Tartaglia. 1996. Leptin and OB-R: Body weight regulation by a cytokine receptor. Cell Growth Factor Rev. 7:303–309.

Widmaier, E. P. 1992. Metabolic feedback in mammalian endocrine systems. Horm. Metab. Res. 24:147–153.

Wilding, J.P.H., S. G. Gilbey, C. J. Bailey, R.A.L. Batt, G. Williams, M. A. Ghatei, and S. R. Bloom. 1993. Increased neuropeptide Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. Endocrinology 132:1939–1944.

Yu, W. H., M. Kimura, A. Walczewska, S. Karanth, and S. M. McCann. 1997. Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci. USA 94:1023–1028.

Zamorano, P. L., V. B. Mahesh, L. M. De Sevilla, L. P. Chorich, G. K. Bhat, and D. W. Brann. 1997. Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinology 65:223–228.

Zhang, B., M. P. Graziano, T. W. Doebber, M. D. Leibowitz, S. White-Carrington, D. M. Szalkowski, P. J. Hey, M. Wu, C. A. Cullinan, P. Bailey, B. Lollmann, R. Frederich, J. S. Flier, C. D. Strader, and R. G. Smith. 1996. Down-regulation of the expression of the obese gene by an antidiabetic thiazolidinedione in Zucker diabetic fatty rats and db/db mice. J. Biol. Chem. 271:9455–9459.

Zhang, F., M. Basinski, J. M. Beals, S. L. Briggs, L. M. Churgay, D. K. Clawson, R. Dimarchi, T. C. Furman, J. E. Hale, H. M. Hsiung, B. E. Schoner, D. P. Smith, X. Y. Zhang, J. Wery, and R. W. Schevitz. 1997. Crystal structure of the obese protein leptin E-100. Nature (Lond.) 387:206–209.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature (Lond.) 372:425–432.

Zhou, Y., M. Shimabukuro, K. Koyama, Y. Lee, M. Wang, F. Trieu, C. B. Newgard, and R. H. Unger. 1997. Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. Proc. Natl. Acad. Sci. USA 94:6386–6390.
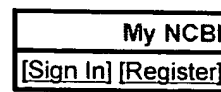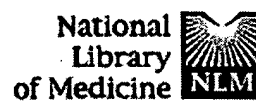
All Databases   PubMed   Nucleotide   Protein   Genome   Structure   OMIM   PMC   Journals   Books

Search PubMed for [   ] Go Clear

Limits   Preview/Index   History   Clipboard   Details

Display Abstract   Show 20   Sort by   Send to

About Entrez

Text Version

All: 1   Review: 0

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut

My NCBI (Cubby)

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov

PubMed Central

☐ **1: Eur J Nucl Med. 1998 Jun;25(6):607-12.**   Related Articles, Links

SpringerLink
FULL-TEXT ARTICLE

Is brain uptake of leptin in vivo saturable and reduced by fasting?

Karonen SL, Koistinen HA, Nikkinen P, Koivisto VA.

Department of Clinical Chemistry, Helsinki University Central Hospital, FIN-00290 Helsinki, Finland.

Leptin is a peptide hormone produced by adipocytes which provides a negative feedback signal to control the amount of body fat. The action of leptin on food intake and weight loss is thought to be mediated by interaction with its hypothalamic receptor. We examined the biodistribution and brain uptake of radioiodinated leptin (123I-leptin) by dynamic gamma imaging in six anaesthetized New Zealand white rabbits. Leptin uptake was seen in the brain, lungs, liver and kidneys. In the brain, increase in radioactivity as a function of time was seen in the choroid plexus area. The choroid plexus to brain radioactivity ratio (CP/BR) was used as the target to background ratio. The CP/BR ratio increased up to approximately 40-60 min, after which a steady state in CP/BR was achieved. The steady state uptake ratio was higher in the rabbits that had fasted for only 6-8 h before the experiment (CP/BR approximately 2.5) than in those that had fasted for 25-27 h before the experiment (CP/BR approximately 1.8). Thus, leptin uptake in vivo occurs in the choroid plexus region of the brain and in the lungs, kidney and the liver. The uptake of leptin in the choroid plexus appears to be saturable, as indicated by the achieved steady state in the CP/BR radioactivity curve 40-60 min following 123I-leptin injection. The lower steady state CP/BR after prolonged fasting may be the result of the downregulation of leptin receptors in the choroid plexus.

PMID: 9618575 [PubMed - indexed for MEDLINE]

Display Abstract   Show 20   Sort by   Send to

pathogenesis of human obesity.

Publication Types:
- Review

PMID: 10714243 [PubMed - indexed for MEDLINE]

Display Abstract ▾ Show 20 ▾ Sort by ▾ Send to ▾

Sep 14 2005 04:34:46

NCBI

Pub Med

National Library of Medicine NLM

My NCBI
[Sign In] [Register]

| All Databases | PubMed | Nucleotide | Protein | Genome | Structure | OMIM | PMC | Journals | Books |

Search PubMed for [   ] Go Clear

Limits   Preview/Index   History   Clipboard   Details

Display Abstract   Show 20   Sort by   Send to

About Entrez

Text Version

All: 1   Review: 1

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Vitam Horm. 2000;59:265-304.

Related Articles, Links

Control of food intake via leptin receptors in the hypothalamus.

Meister B.

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Food intake is regulated via neural circuits located in the hypothalamus. During the past decade our knowledge on the specific mediators and neuronal networks that regulate food intake and body weight has increased dramatically. An important contribution to the understanding of hypothalamic control of food intake has been the characterization of the ob gene product (leptin) via positional cloning. Absence of circulating, functionally active, leptin hormone results in massive obesity as seen in ob/ob mice. Leptin inhibits food intake and increases energy expenditure via an interaction with specific leptin receptors located in the hypothalamus. Leptin receptors, of which there are several splice variants (Ob-Ra through Ob-Re), belong to the superfamily of cytokine receptors, which use the JAK-STAT pathway of signal transduction. Obese db/db mice, which have a mutation in the db locus, are unable to perform JAK-STAT signal transduction due to absence of functionally active (long form; Ob-Rb) leptin receptors. Ob-Rb is primarily expressed in the hypothalamus, with particularly high levels in the arcuate, paraventricular, and dorsomedial nuclei and in the lateral hypothalamic area. The abundance of leptin receptors in the ventromedial and lateral hypothalamus supports early observations that these two regions are intimately associated with the regulation of food intake. Leptin receptors have been identified in neuropeptide Y (NPY)/lagouti-related peptide (AgRP)- and proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART)-containing neurons of the ventromedial and ventrolateral arcuate nucleus, respectively, and in melanin-concentrating hormone (MCH)- and hypocretin/orexin-containing neurons of the lateral hypothalamus, suggesting that the above-mentioned messengers are mediators of leptin's action in the hypothalamus. Indeed, functional studies show that NPY, AgRP, POMC-derived peptides, CART, MCH, and hypocretins/orexins all are important regulators of food intake. Leptin is essential for normal body weight balance, but the exact mechanisms by which leptin activates hypothalamic neuronal circuitries is known to a limited extent. In order to find pharmaceutical approaches to treat obesity, further studies will be needed to reveal the exact mechanisms by which leptin lowers body weight and which role leptin and leptin receptors have in the

THIRTEENTH EDITION

HARRISON'S
PRINCIPLES
OF
INTERNAL
MEDICINE

Editors

**KURT J. ISSELBACHER, A.B.,
M.D.**

Mallinckrodt Professor of Medicine, Harvard Medical
School; Physician and Director, Cancer Center,
Massachusetts General Hospital, Boston

**EUGENE BRAUNWALD, A.B.,
M.D., M.A. (Hon.), M.D. (Hon.), Sc.D. (Hon.)**

Hersey Professor of the Theory and Practice of Medicine,
Harvard Medical School; Chairman, Department of
Medicine, Brigham and Women's Hospital, Boston

JEAN D. WILSON, M.D.

Charles Cameron Sprague Distinguished Chair and
Professor of Internal Medicine; Chief, Division of
Endocrinology and Metabolism, The University of Texas
Southwestern Medical Center, Dallas

**JOSEPH B. MARTIN, M.D., Ph.D.,
F.R.C.P. (C), M.A. (Hon.)**

Professor of Neurology and Chancellor, University of
California, San Francisco

ANTHONY S. FAUCI, M.D.

Director, National Institute of Allergy and Infectious
Diseases; Chief, Laboratory of Immunoregulation; Director,
Office of AIDS Research, National Institutes of Health,
Bethesda

DENNIS L. KASPER, M.D.

William Ellery Channing Professor of Medicine, Harvard
Medical School; Chief, Division of Infectious Diseases,
Beth Israel Hospital; Co-Director, Channing Laboratory,
Brigham and Women's Hospital, Boston

Note: Dr. Fauci's work as editor and author was performed outside the scope of his employment by the U.S. government. This work represents his personal and professional views and not necessarily those of the U.S. government.

Thirteenth Edition

34567890 DOW DOW 98765

FIGURE 68-6 Sympathetic nervous system effects on blood pressure. Sympathetic stimulation (+) increases blood pressure by effects on the heart, the veins, the kidneys, and the arterioles. The net result of sympathetic stimulation is an increase in both cardiac output and peripheral resistance. AII = angiotensin II. *[From JB Young, L Landsberg, in P Sleight et al (eds), Scientific Foundations of Cardiology, London, Heinemann, 1981.]*

Whether primary sympathetic overactivity plays a role in the pathogenesis of essential hypertension is uncertain owing to the insensitivity of currently available methods of assessing regional sympathetic activity in humans. It is well established, however, that the sympathetic nervous system plays at least a permissive role in hypertension. Despite the elevated blood pressure, sympathetic nervous system activity is not suppressed in hypertensive patients, and reflex control of the circulation is retained, due in part to upward resetting of the baroreceptors. In addition, peripheral sensitivity of the vasculature to NE is either normal or enhanced. The maintenance of sympathetic nervous system activity in patients with hypertension accounts for the hypotensive effects of antiadrenergic agents.

During antihypertensive treatment with vasodilators or diuretics, the sympathetic nervous system may be activated in response to decreased pressure in either the venous or arterial circulation (Fig. 68-3). The heightened sympathetic activity that results, in addition to causing tachycardia, may oppose the antihypertensive therapy by activating the various effector systems shown in Fig. 68-6. Agents with antiadrenergic effects, therefore, have a fundamental role in the therapy of most hypertensive patients.

ANGINA PECTORIS (See also Chap. 203)   Sympathetic stimulation of the cardiovascular system increases myocardial oxygen consumption as a consequence of elevated heart rate, enhanced myocardial contractility, and increased myocardial wall tension. Attacks of angina, therefore, are often precipitated by situations associated with sympathetic activation such as exercise, eating, and cold exposure. Beta blockade is beneficial in the treatment of angina because of reduction in sympathetic stimulation of the heart. Alpha-adrenergically mediated coronary vasoconstriction also may contribute to coronary spasm.

HYPERTHYROIDISM (See also Chap. 334)   Many of the peripheral manifestations of hyperthyroidism suggest a hyperadrenergic state. Enhancement of beta-receptor responses in hyperthyroidism is due in part to effects on the beta receptor. Thyroid hormone, in some tissues and in some species, increases receptor number; in other tissues, even when beta-receptor number is not increased, coupling of receptor occupancy to the adenylate cyclase cyclic-AMP system is augmented to amplify catecholamine-induced responses. Since thyroid hormone excess does not suppress sympathetic nervous system activity (plasma NE levels are normal in thyrotoxic patients), a "normal" level of sympathetic activity may evoke an exaggerated physiologic response. Many of the adrenergic manifestations of hyperthyroidism are diminished by treatment with beta-receptor blocking agents.

ORTHOSTATIC HYPOTENSION The maintenance of blood pressure during upright posture depends on an adequate blood volume, an unimpaired venous return, and an intact sympathetic nervous system. Significant postural hypotension, therefore, often reflects extracellular fluid volume depletion or dysfunction of the circulatory reflexes. Diseases of the nervous system, such as tabes dorsalis, syringomyelia, or diabetes mellitus, may disrupt these sympathetic reflexes with resultant orthostatic hypotension. Although any antiadrenergic agent may impair the postural sympathetic response, orthostatic hypotension is most prominent with drugs that block neurotransmission within the ganglia or adrenergic neurons.

The term *idiopathic orthostatic hypotension* refers to a group of degenerative diseases involving either the pre- or postganglionic sympathetic neurons. Involvement of the postganglionic sympathetic nervous system is characterized by low basal NE levels, while involvement at the level of the central nervous system or preganglionic sympathetic neurons is associated with normal basal plasma NE levels. In both cases, the plasma NE response to upright posture is deficient. Orthostatic hypotension caused by disruption of preganglionic autonomic neurons within the intermediolateral column of the spinal cord often occurs in association with degenerative changes of basal ganglia and other portions of the central nervous system. In the latter situation, known as *multiple-systems atrophy*, the *Shy-Drager syndrome*, orthostatic hypotension occurs along with a variety of neurologic disturbances, including Parkinson's disease.

Treatment of orthostatic hypotension is usually unsatisfactory except in the mildest cases. There is no way of reestablishing the normal relationship between fall in venous return and sympathetic neuronal activation. Volume expansion with fludrocortisone and a liberal salt diet in conjunction with fitted stockings to the waist, as well as elevation of the head of the bed to avoid recumbency, will maintain plasma volume and venous return and frequently provide symptomatic improvement. Rarely a beneficial response may be obtained from treatment with sympathomimetic amines (including clonidine).

PHARMACOLOGY OF THE SYMPATHOADRENAL SYSTEM

A variety of therapeutic agents affect sympathetic nervous system function or interact with adrenergic receptors, making it possible to stimulate or suppress effects mediated by catecholamines with some degree of specificity (Table 68-1).

SYMPATHOMIMETIC AMINES Sympathomimetic amines may directly activate adrenergic receptors (direct acting) or release NE from the sympathetic nerve endings (indirect acting). Many agents have both direct and indirect effects.

Epinephrine and norepinephrine The naturally occurring catecholamines act predominantly by the direct stimulation of adrenergic receptors. NE is employed to support the circulation and elevate the blood pressure in hypotensive states (Chap. 34). Peripheral vasoconstriction is the major effect, although cardiac stimulation occurs as well. E, also employed as a pressor, has special usefulness in the treatment of allergic reactions, especially those associated with anaphylaxis. E antagonizes the effects of histamine and other mediators on vascular and visceral smooth muscle and is useful in the treatment of bronchospasm.

Dopamine *Dopamine* is used in treating hypotension, shock (Chap. 34), and certain forms of heart failure (Chap. 195). At low infusion rates it exerts a positive inotropic effect both by a direct action on the cardiac $beta_1$ receptors and by the indirect release of NE from sympathetic nerve endings in the heart. At low doses direct stimulation of dopaminergic receptors in the renal and mesenteric vasculature also results in vasodilation in the gut and kidney and facilitates sodium excretion. At higher infusion rates interaction with alpha-adrenergic receptors results in vasoconstriction, an increase in peripheral resistance, and an elevation of blood pressure.
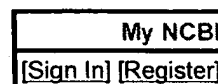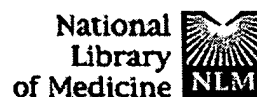
NCBI

Pub Med

National Library of Medicine NLM

My NCBI
[Sign In] [Register]

All Databases   PubMed   Nucleotide   Protein   Genome   Structure   OMIM   PMC   Journals   Books

Search PubMed ▼ for [   ] Go Clear

Limits   Preview/Index   History   Clipboard   Details

Display Abstract ▼ Show 20 ▼ Sort by ▼ Send to ▼

About Entrez

Text Version

All: 1   Review: 0

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Acta Neurol Scand. 1988 May;77(5):387-96.   Related Articles, Links

Adrenaline-induced hypertension: morphological consequences of the blood-brain barrier disturbance.

Sokrab TE, Johansson BB, Tengvar C, Kalimo H, Olsson Y.

Department of Neurology, University of Lund, Sweden.

Acute hypertension may transiently open the blood-brain barrier (BBB). To determine whether such temporary exposure of the brain parenchyma to plasma constituents may lead to permanent morphological alterations, acute hypertension was induced by i.v. adrenaline in conscious rates given Evan's blue and horseradish peroxidase as tracers. The brain were perfused in situ 24 h later: 17 of 21 brains showed multifocal sites of extravasation of the tracers and of endogenous plasma albumin, fibrinogen and fibronectin identified by immunohistochemistry. The proteins spread locally in the parenchyma and were taken up by neurons. Within the leaking sites in the cortex, hippocampus, thalamus and basal ganglia some shrunken and grossly distorted acidophilic neurons were present. Focal areas of sponginess were observed in the subpial and subependymal zones. Thus, a transient opening of the BBB may lead to neuronal damage.

PMID: 3414376 [PubMed - indexed for MEDLINE]

Display Abstract ▼ Show 20 ▼ Sort by ▼ Send to ▼

Oct 6 2005 04:38:59